THE AMERICAN
JOURNAL OF PHYSIOLOGY

VOL. 111 MARCH 1, 1935 No. 2

PERMEABILITY OF THE RED CELL MEMBRANE TO GLUCOSE[1]

KALMEN A. KLINGHOFFER

From the Department of Internal Medicine, Yale University, New Haven, Conn.

Received for publication November 17, 1934

The free permeability of the human red cell membrane to glucose *in vivo* has been adequately demonstrated, but certain apparent contradictions in its behavior *in vitro* require further elucidation. These contradictions were noted by Masing in 1914 (7): when iso-osmotic solutions of glucose or sodium chloride were added to blood, glucose analyses revealed the cell membrane to be freely permeable to that substance; when cells were placed in a solution of iso-osmotic glucose, at 38°C., lysis was not complete in 5 hours. Masing's findings have been corroborated, but not explained, by later investigators. Since the permeability of red blood cells to glucose has been a source of continuous controversy, it was decided to review the subject in an endeavor to ascertain the reasons for the confusion of opinion. For comparative purposes, the actions of water, urea, glycerol and sucrose, as well as those of glucose, were studied.

EXPERIMENTAL PROCEDURES. The permeability of the red cell membrane can be readily determined osmotically, an increase in the volume of the cell indicating that either solvent or solution is entering the cell. If the solution added is iso-osmotic with serum, both solute and solvent must penetrate the membrane if the corpuscular volume is to increase. In a comparative series the increase of corpuscular volume can be used as an indication of cellular penetration of the solute.[2]

The procedures followed were two. The first consisted merely, after a drop of blood had been added to 2 cc. of the solution to be tested, of noting the presence or absence of hemolysis.

In the second, the solution was added to a portion of the blood, and the

[1] This article represents work done in fulfillment of the thesis requirement for the degree of Doctor of Medicine at Yale University School of Medicine.

[2] [illegible]ill be shown, in the experiments here reported, that the concentrations of [illegible]nces leaving the cells are negligible.

change in the volume of the cells determined by means of the hematocrit. The change in the oxygen capacity was used as an indication of the extent of dilution of the blood. From a knowledge of the amounts of water originally in the cells and serum, the percentage changes of the water of cells and serum were calculated. The amount of water originally in the cells was estimated with the aid of the following equation:

$$\text{(a)}\quad \text{Water of cells in per cent} = 109.2 - 4.6 - \left(\frac{\text{Oxygen capacity}}{1.34} \times \frac{100}{\text{Cell volume}}\right);$$

109.2 being the average specific gravity of human red blood cells, and 4.6 being the average weight in grams of the solids other than hemoglobin in 100 cc. of blood (4). The serum was considered to contain 93.5 per cent of water (by volume) (4). The percentage change of water of cells was calculated by the equation:

$$\text{(b)}\quad \text{Percentage increase of water of cells} = \frac{b\,(1000 + a) - 1000\,c}{cr};$$

b representing the volume of cells in 1000 cc. of diluted blood, c the volume in 1000 cc. of undiluted blood; a, the volume of solution added to 1000 cc. of the undiluted blood, and r the per cent of water in the cell. A similar equation was employed in calculating the percentage change of water of serum.[3]

In certain cases other determinations, to be described, were done. Defibrinated human venous blood was used in all cases. Unless otherwise stated, it was kept at room temperature for the duration of the experiment.

ANALYTICAL METHODS. *Oxygen capacities* were determined by the carbon monoxide method of Van Slyke and Hiller (13). In a few cases the blood was saturated with air, and the oxygen content determined by the absorption method described by Van Slyke and Neill (14), with the anthraquinone beta-sulfonate catalyst added to the hydrosulfite.

Cell volumes were determined by the hematocrit method. The hematocrits consisted of 0.5 mm. bore calibrated glass tubing, 5.0 cm. long. These were centrifuged in an International Instrument Company centrifuge, type B, size 1, at 1400 r.p.m. until constant volume was attained, usually about 1 hour.

Sodium and potassium were determined by methods described by Hald (5).

[3] The possible error of the results varies with several factors. In general, it tends to be greater (in per cent) in those cases in which the changes in volume are small, and less when the volume changes are great. If the changes are less than 10 per cent the possible error may reach 30 per cent; if the changes are more than 50 per cent, the error is probably less than 5 per cent.

Chloride was determined by the micro method of Patterson (11), with the modification of digestion with potassium permanganate before titration.

Glucose was determined with Somogyi's filtrate (12) and Benedict's reagent (1).

TABLE 1

Results obtained when 1 drop of blood was added to 2 cc. of solutions containing varying concentrations of various solutes

Readings were taken immediately. The presence of hemolysis is indicated by +; its absence by 0.

UREA, PER CENT	SODIUM CHLORIDE, PER CENT							POTASSIUM CHLORIDE			GLUCOSE		
	1.1	0.9	0.7	0.55	0.45	0.40	0.36	0.8	0.62	0.57	2.7	2.0	0
3.0				0	0			0	0	+	0	+	+
2.5				0		0	+						+
2.0	0	0	0	0	0	0	+	0	0	+	0	+	+
1.5	0	0	0				+						+
1.0	0	0	0	0	0	0	+	0	0	+			+
0.5	0	0	0	0	0	0	+	0	0	+	0	+	+
0	0	0	0	0	0	0	+	0	0	+	0	+	+
GLYCEROL, PER CENT													
4.0			0		0	+				+		+	+
2.9			0		0	+				+		+	+
2.0			0		0	+				+		+	+
1.4			0		0	+				+		+	+
0			0		0	+				+		+	+

SUBJECT	GLUCOSE, PER CENT				
	3.0	2.7	2.5	2.3	2.1
A	0	0	0	+	+
B	0	0	0	+	+
C	0	0	0	0	+
D	0	0	+	+	+
E	0	0	+	+	+
F	0	0	+	+	+
G	0	0	+	+	+
H	0	0	0	0	+
I	0	0	0	+	+

RESULTS AND DISCUSSION. One drop of blood was added to 2 cc. of the solutions indicated in table 1, and the presence or absence of hemolysis noted. In those cases in which hemolysis occurred in the solutions containing glycerol there was a delay of several seconds; otherwise urea and

glycerol apparently did not differ from water in their hemolytic actions, lysing human erythrocytes unless the concentration of salt or glucose was high enough to prevent lysis. The concentrations of urea and glycerol had no detectable effect on the results.

Table 2 shows that glucose in concentration above 2.3 per cent did not cause hemolysis, and was as effective as 0.45 per cent sodium chloride or

TABLE 2

Results obtained when 1 drop of blood was added to 2 cc. of solutions of glucose and sodium chloride

The absence of hemolysis is indicated by 0, the presence by 1+, 2+, 3+ and 4+, 4+ indicating complete hemolysis.

GLUCOSE	SODIUM CHLORIDE	TOTAL EQUIVALENTS* IN TERMS OF		TIME, MINUTES						
		Sodium chloride	Glucose	0	5	10	15	20	25	30
per cent	*per cent*	*per cent*	*per cent*							
1.37	0.25	0.48	2.69	0	0	1+	1+	2+	3+	4+
1.37	0.23	0.46	2.58	0	0	4+	4+	4+	4+	4+
1.37	0.20	0.43	2.43	0	0	4+	4+	4+	4+	4+
1.37	0.18	0.41	2.32	0	1+	4+	4+	4+	4+	4+
1.37	0.15	0.38	2.16	1+	3+	4+	4+	4+	4+	4+
1.37	0.13	0.36	2.06	3+	4+	4+	4+	4+	4+	4+
1.37	0.10	0.33	1.90	4+	4+	4+	4+	4+	4+	4+
0.88	0.30	0.49	2.57	0					3+	4+
1.21	0.25	0.48	2.54	0					1+	3+
1.49	0.20	0.48	2.55	0					0	1+
1.79	0.15	0.49	2.59	0					0	0
2.09	0.10	0.49	2.61	0					0	0
2.50	0	0.47	2.50	0					0	0
2.30	0	0.43	2.30	0					0	0
2.10	0	0.40	2.10	1+					1+	1+
1.90	0	0.36	1.90	4+					4+	4+
0	0.42	0.42	2.22	1+					1+	1+
0	0.40	0.40	2.11	2+					3+	3+
0	0.40	0.38	2.00	3+					4+	4+
0	0.36	0.36	1.90	4+					4+	4+

* It is assumed that 0.1 per cent sodium chloride is equivalent osmotically to 0.53 per cent glucose.

0.6 per cent potassium chloride in preventing hemolysis in urea and glycerol solutions. Below a concentration of 2.3 per cent, however, hemolysis occurred, unless the concentration of sodium or potassium chloride present was sufficient of itself to prevent it. The anti-hemolytic actions of salt and glucose were not identical; unless either prevented hemolysis, both together failed to do so for more than a limited period.

TABLE 3

Percentage increases of water of cells and serum when water, 1.86 per cent urea and 2.9* per cent glycerol were added to blood*

DILUTION	PERCENTAGE INCREASES OF WATER OF		TIME
	Cells	Serum	
Water added			
per cent	*per cent*	*per cent*	*minutes*
12	11	17	15
	11	17	50
	16	14	180
17	17	23	11
	14	26	120
20	20	26	240
20	21	27	120
22	30	25	240
23	21	32	15
	21	33	50
	29	27	180
24	22	34	120
25	26	32	15
	28	31	110
34	36	46	15
	37	45	50
	44	41	180
35	34	49	11
	33	50	120
38	30	58	300
50	55	58	8
	54	58	128
71	74	85	8
	Hemolysis		128
Urea solution added			
18	12	28	15
	11	29	90
	9	31	270
24	23	31	15
	23	31	110
37	29	55	15
	30	55	90
	25	57	270
56	62	74	20
	50	84	90
83	Hemolysis		20
Glycerol solution added			
50	53	65	10
	53	65	120
50	45	62	10
	57	59	130
71	79	72	10
	71	83	130

* 1.86 per cent urea and 2.9 per cent glycerol are iso-osmotic with 0.9 per cent sodium chloride.

There was no hemolysis in the solution of sucrose tested, 9.2 per cent.

In the subsequent experiments the second procedure was followed, the various solutions being added to the supernatant serum after the blood had been centrifuged in round bottomed tubes at 1400 r.p.m. for 20 minutes. It will be noted (table 3) that water, urea solution and glycerol solution tended to distribute themselves immediately, and almost equally (in per cent) in the water of cells and serum, until the percentage increase of water of the cells approximated 80, when hemolysis occurred. The increase in cellular water was rapid, i.e., occurred in less than 20 minutes,[4] but the percentage increase tended to be less than that of the serum water. If the findings in the experiments in which water was added are averaged, it is

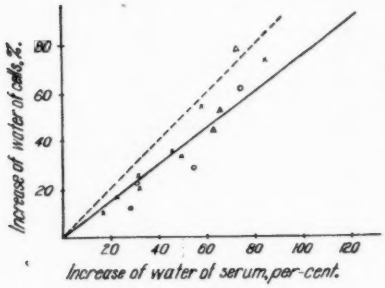


Fig. 1

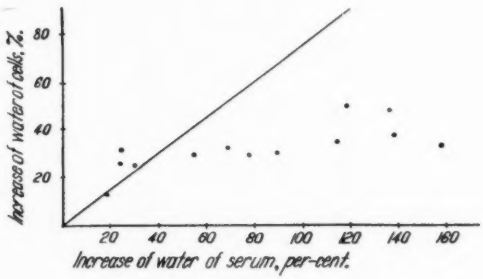


Fig. 2

Fig. 1. Percentage increases in water of cells and serum when water, x, 1.86 per cent urea, 0, or 2.9 per cent glycerol, Δ, was added to blood. The broken line marks an angle of 45°; the solid line, describing the average curve of the observed points, marks an angle of 36° with the abscissa.

Fig. 2. Percentage increases of water of cells and serum when 5.5 per cent glucose was added to blood. The solid line, placed at an angle of 36° with the abscissa, represents the effect of the addition of equal amounts of water.

found that $x/y = 1.35$ (x representing the percentage increase of serum water, y the percentage increase of cell water), and not 1 as would be expected if the membrane were freely permeable, and the total water content of the cell osmotically "free." Plotted one secures a line forming an angle of 36°, rather than 45°, with the abscissa (fig. 1). There can be little doubt, however, that the membrane *is* freely permeable to water. The explanation of the discrepancy is not strictly germane to the subject of this paper. In the other experiments the results of dilution with water are used as criteria of free permeability.

It is seen (table 4 and fig. 2) that when less than 2 parts of 5.5 per cent

[4] It was usually impossible to ascertain the cell volume in less than 20 minutes; a few determinations, however, indicate that the increase occurred within a few minutes.

glucose were added to 5 of blood, the distribution of water (and presumably of glucose) between the corpuscular and serum water was immediate and almost equal, as it had been when the diluting fluid was water. Above a

TABLE 4

Percentage increases of water of cells and serum when 5.5 per cent glucose was added to blood

Equilibrium is considered established in those cases in which the increases are printed in italics.

DILUTION	PERCENTAGE INCREASES OF WATER OF		TIME	GLUCOSE, ESTIMATED
	Cells	Serum		
per cent	*per cent*	*per cent*	*minutes*	*mgm. per cent*
7	*9*	*9*	180	475
13	*13*	*19*	15 and 175	750
20	*26*	*24*	10 and 120	1000
22	*31*	*25*	15 and 240	1075
24	*25*	*30*	15 and 240	1100
28	*27*	*37*	105	1300
35	*29*	*55*	12 and 180	1550
47	32	69	15	
	45	*63*	120	1850
48	*48*	*62*	180	1875
49	29	78	10	1900
	51	*65*	150	
56	30	90	15	2075
	51	77	120	
70	35	115	15	
	74	*91*	240	2350
74	*72*	*98*	180	2450
76	50	119	15	2475
	68	103	180	
80	64	112	120	2550
83	48	137	20	2600
84	37	139	15	2600
	67	121	120	
86	72	127	180	2650
91	76	123		2700
92	33	158	15	2750
	61	141	150	
	85	126	450	
93	78	127	150	2750
101	83	141		2850
106	74	166		2900
124	90	169		3150

dilution of about 40 per cent, however, the water of the cells did not continue to increase in proportion to the water of the serum, and there was no hemolysis.

The experiments already cited indicate very well the nature of the confusion in the literature concerning the diffusibility of glucose. In conditions approaching the physiological, that is, when small amounts of glucose solution are added to blood, the red cell membrane is freely permeable to glucose, as it is *in vivo*; at higher concentrations of glucose it is relatively impermeable.

Before discussing this further, attention is called to table 4, in which at higher concentrations the cellular volume is seen to increase gradually over a period of several hours, without the appearance of hemolysis. Ege (2) has shown that such an increase cannot be ascribed to glycolysis. Furthermore, in one experiment 1 part of 5.5 per cent glucose solution was added to 5 of blood, and cell volumes were determined at intervals for 22 hours, during part of which time the blood was kept at 10°C. In 22 hours there was no change in the corpuscular volume, the glucose content remaining above 0.8 per cent. Neuwirth (10) has recently reported the changes in glucose content of hyperglycemic blood standing at room temperature. It is questionable whether sufficient quantities of lactic acid could be produced from the small amounts of glucose which disappeared to alter the corpuscular volumes appreciably. To test the possibility further, in one case blood to which nothing had been added was incubated for 4 hours at 38°C.; the volume of the cells increased only from 44.9 to 46.1 per cent. Since in most of the experiments described in this paper the blood was kept at room temperature the glycolysis must have been less than that in the experiment just mentioned. There is additional evidence that the gradual increase in volume was consequent upon the gradual penetration of glucose. When a drop of blood was added to 2 cc. of solutions of 5.5 and 16.5 per cent glucose, and the solutions were incubated at 38°C. for 5 hours, complete hemolysis was found in the weak solutions, while in the more concentrated solutions hemolysis was minimal.

Ege (2) found that when cells were suspended in 5.5 per cent glucose, although the distribution ratio

$$\frac{\text{Glucose of cell water}}{\text{Glucose of serum water}} \text{ equalled 1,}$$

the volume of the cells was 50 per cent greater than the volume of the same cells suspended in 0.9 per cent sodium chloride.[5] Applying the equation

$$P(V - X) = P_1(V_1 - X),$$

in which P represents the osmotic pressure of the control solution, V the volume of the cells in the control solution, X the disperse phase, P_1 the

[5] Actually Ege found the ratio $\frac{\text{Glucose of cells}}{\text{Glucose of serum}}$ equal to 0.8.

osmotic pressure of the tested solution (in this case glucose solution), and V_1 the volume of the cells in the tested solution (in this case 150 if V equalled 100), he concluded that the cell contained glucose in "solution" in a concentration only half as great as that of the extra-cellular fluid, and that, therefore, the remainder of the glucose, which appeared from the analyses to be in the cells, was probably adsorbed. This equation is not applicable, however, for it neglects the osmotic pressure of the glucose which penetrated the cell membrane. Employing the equation

$$\frac{P}{V - X} = \frac{P_1 \times G}{V_1 - X}$$

in which G represents the osmotic pressure of the glucose in the cell, and solving for X, one obtains a value of 34 for the disperse phase, which is fairly close to the known value. Furthermore, analyses of serum, even at high dilutions and within 15 minutes of the addition of the glucose solution, reveal concentrations indicative of an equal distribution in the water of cells and serum. Some, of course, could have been adsorbed, but it is quite unlikely that the amount adsorbed and the amount dissolved in the cell would together always equal the amount which it is estimated would be dissolved in the cell if the distribution ratio between the water of the cells and that of the serum equalled 1. Ege (3), himself, has more recently discarded the theory of adsorption as untenable, for he found 2 washings with saline sufficient to remove all the glucose from the cells.

Ege (2) thought that serum accelerated the diffusion of glucose through the cell membrane. Because, in the experiment above in which the diffusion was inhibited, the serum was greatly diluted, another series of experiments was conducted. Blood was diluted from 45 to 100 per cent with 0.9 per cent sodium chloride, and the increase of the water of cells and serum on the subsequent addition of 5.5 per cent glucose was noted. (Controls using urea solution were also run.) The results obtained were similar to those presented in table 4, and indicated that dilution of serum alone does not alter the permeability of the red cell membrane to glucose. Similarly the possibility that dilution of electrolytes affected the permeability of the membrane was tested by diluting blood from 45 to 95 per cent with 9.2 and 10.6 per cent sucrose solution before adding glucose solution. Again there was no alteration in the final results; nor did an increase in the concentration of electrolytes, secured by the addition of 1.35 per cent sodium chloride, change the nature of the curves obtained.

The results presented in table 5 indicate, moreover, that something other than mere dilution was involved, for the use of more concentrated glucose solutions was sufficient to change both the rate and the extent of the increase in corpuscular volume.

If glucose were the only factor affecting the diffusion of glucose through

the cell membrane, one would expect the increase in cellular water to be immediate and almost equal to that of the serum water as long as the glucose concentration were less than 2.3 per cent, for it was below this concentration that hemolysis occurred in the first series of experiments. But estimations of the glucose concentrations in the second series do not support

TABLE 5

Percentage increases of water of cells and serum when glucose solutions of varying concentrations were added to blood

Equilibrium is considered established in those cases in which the increases are printed in italics.

DILUTION	PERCENTAGE INCREASES OF WATER OF		TIME	GLUCOSE, ESTIMATED
	Cells	Serum		
	per cent	*per cent*	*minutes*	*mgm.*
23 per cent, 16.5 per cent glucose	−6	52	25	3000
	+11	39	155	
	+22	*32*	500	
40 per cent, 16.5 per cent glucose	−9	87	25	4800
	−5	84	155	
	+24	65	500	
24 per cent, 11.0 per cent glucose	8	42	15	2250
	17	36	100	
	29	*29*	450	
25 per cent, 11.0 per cent glucose	*27*	*35*	120	2300
31 per cent, 11.0 per cent glucose	21	48	180	2750
34 per cent, 11.0 per cent glucose	10	49	15	2900
	32	*42*	270	
48 per cent, 11.0 per cent glucose	31	83	240	3500
50 per cent, 11.0 per cent glucose	27	77	120	3750
50 per cent, 11.0 per cent glucose	*48*	*69*	300	3750
	54	63	363	
90 per cent, 11.0 per cent glucose	−19	187	15	5300
	−1	175	100	
	+28	157	450	
100 per cent, 2.8 per cent glucose	*94*	*139*	10	1450
100 per cent, 2.2 per cent glucose	*86*	*146*	10	1150
100 per cent, 1.8 per cent glucose	*89*	*142*	10	950
130 per cent, 1.8 per cent glucose	Hemolysis		5	635
100 per cent, 1.2 per cent glucose	Hemolysis		10	650

this assumption, for the gradual increase in corpuscular volume was present whenever the glucose concentration exceeded 1.5 per cent, and it was impossible to cause hemolysis with any dilution if the glucose concentration exceeded 0.9 per cent (table 5). Experiments in which dry glucose was added to whole blood did not clarify matters any, for under these conditions a change in the membrane permeability was apparent at about 2.0 per cent.

Consequently, although at present the cause of the inhibition of the cellular penetration of glucose can not be attributed to any factor other than glucose, obviously other factors are involved.

There are several possibilities as to the nature of these specific actions, or rather, of their *modi operandi*. They cannot be ascribed merely to a toughening of the membrane, for as Jacobs (6) has shown, the membrane is certainly not able to resist large osmotic forces. There is a further possibility that something leaves the cell, lowering the osmotic pressure

TABLE 6

Changes in chloride and base concentrations of serum when solutions of glucose were added to blood

In the first two experiments the blood had previously been diluted with 9.2 per cent sucrose, 5.5 per cent glucose was added in all except the last experiment, in which 11.0 per cent was used.

DILUTION	CONTROL SERUM	DILUTED SERUM	DILUTED SERUM, CALCULATED*	TIME
		Chloride, milli-equivalents		
				minutes
20	40.1	37.4	33.0	10
49	38.4	26.1	24.5	5
84	99.9	45.7	47.1	120
100	102.3	40.5	41.6	15
		Sodium, milli-equivalents		
28	132.4	96.0	98.2	180
86	136.5	62.4	62.5	180
50	134.7	77.7	78.4	120
		Potassium, milli-equivalents		
86	6.21	2.84	2.84	180
93	7.42	3.29	3.40	150

* Calculated on the assumption that no chloride, sodium or potassium ions crossed the cell membrane.

within. Analyses of serum in a number of cases proved that neither chloride nor base[6] diffused out of the cell under the conditions of these experiments (table 6).[7] Mond (8) suggested that the aldehyde grouping

[6] Most of the base determinations were done by Miss Pauline M. Hald.

[7] It has been claimed (8, 9) that in solutions of non-electrolytes base is lost by the red cell to the surrounding medium. In addition to the experiments mentioned, analyses were made in a number of experiments in which sucrose solution was added to serum. Except in the two experiments performed with the blood of one subject, it was found that no base crossed the cell membrane. In the two experiments referred to, the amounts lost by the cells were not osmotically significant.

of the glucose molecule had some specific effect on the cell membrane, but dilution of serum with lactose previous to dilution with glucose failed to support the suggestion. As the results obtained did not differ from those obtained when the initial diluting solution was sucrose, the aldehyde complex cannot be considered a pertinent factor.

SUMMARY

The permeability of the human red cell membrane was studied osmotically.

The addition of a drop of blood to 2 cc. of glucose solution revealed that the membrane was freely permeable to glucose below a concentration of 2.3 per cent.

When less than 2 parts of glucose solution were added to 5 of blood the increase in corpuscular volume was comparable to that obtained when water or urea solution was added, being immediate and almost equal in per cent to that of the serum volume. When more than 2 parts were added, however, a relative impermeability existed, the cells increasing in volume much less than they increased on adding the same amount of water. The change in permeability of the cell membrane was found not to be due to a dilution of the serum or of the serum electrolytes. The change is intimately associated with, but not entirely due to the increase in the glucose concentration.

No evidence was obtained that augmentation of the concentration of non-electrolytes in the serum affected the permeability of the red cell membrane to base, and in the experiments here reported neither base nor chloride was lost by the cells.

REFERENCES

(1) Benedict, S. R. J. Biol. Chem. **68:** 759, 1926.
(2) Ege, R., E. Gottlieb and N. W. Rakestraw. This Journal, **72:** 76, 1925.
(3) Ege, R. and J. Roche. Compt. rend. Soc. de biol. **101:** 98, 1928.
(4) Eisenman, A. J. Unpublished studies.
(5) Hald, P. M. J. Biol. Chem. **103:** 471, 1933.
(6) Jacobs, M. H. Biol. Bull. **62:** 185, 1932.
(7) Masing, E. Pflüger's Arch. **149:** 227, 1912.
(8) Mond, R. Pflüger's Arch. **217:** 618, 1927.
(9) Netter, H. Pflüger's Arch. **220:** 107, 1928.
(10) Neuwirth, I. J. Biol. Chem. **104:** 129, 1934.
(11) Patterson, J. Biochem. J. **22:** 758, 1928.
(12) Somogyi, M. J. Biol. Chem. **86:** 655, 1930.
(13) Van Slyke, D. D. and A. Hiller. J. Biol. Chem. **78:** 807, 1928.
(14) Van Slyke, D. D. and J. M. Neill. J. Biol. Chem. **61:** 523, 1924.

THE RÔLE OF THE NERVOUS SYSTEM IN THE REGULATION OF THE GLYCOGEN METABOLISM OF SKELETAL MUSCLE

H. M. HINES AND G. C. KNOWLTON

From the Department of Physiology, State University of Iowa

Received for publication November 24, 1934

There is but little conclusive evidence available concerning the rôle of the nervous system in the regulation of the static glycogen metabolism of skeletal muscle. Recent investigations have been concerned chiefly with glycogen breakdown during muscular contraction and glycogen resynthesis during subsequent recovery periods, with relatively little attention being given to the extent to which the nervous system participates in the regulation of glycogen synthesis and breakdown in non-exercised muscle.

In this report are described the characteristic changes which occur in the glycogen metabolism of the gastrocnemius muscle of the rat at various intervals of time following section of the sciatic nerve. Three general aspects of glycogen metabolism have been studied. First the glycogen concentration of normal control muscle was compared to the glycogen concentration of denervated muscle at various periods of time after operation. In a second series of experiments the ability of muscles, which had been deprived of their nerve supply for various periods of time, to increase their glycogen concentration was tested. Two types of experiments were made. In one the effects of forced glucose ingestion upon the glycogen concentration of normal and denervated muscles of well fed animals were observed. In the other glucose was given to animals whose glycogen stores had previously been reduced by either fasting or the administration of either insulin or adrenalin. A third series of experiments was concerned with a comparison of the effects of such glycogenolytic agencies as fasting, the administration of insulin, adrenalin and thyroxin upon the glycogen concentration of control and denervated muscles.

METHODS. A group of 158 full-grown rats served as experimental animals. In all experiments the normally innervated muscle of the opposite limb served as the control. The methods employed for nerve section, muscle removal and glycogen analysis have been described elsewhere (Knowlton and Hines, 1934). We have adopted a rigid standardization of the conditions of the experiments which are required according to Macleod (1934) in order to approximate the true glycogen concentration of tissues under various conditions.

In the experiments involving fasting the animals were kept in individual metabolism cages for the designated periods. Water was allowed ad libitum in all experiments. Glucose was administered by stomach tube as a 50 per cent solution, in amounts of 0.75 gram per 100 grams body weight, 3 hours before tissue removal. Synthetic thyroxin, in amounts of 0.7 mgm., was administered subcutaneously on the fifth and sixth days after denervation. Unless otherwise indicated in the tables, the adrenalin and insulin were administered subcutaneously in amounts of 0.02 mgm. and one unit respectively per 100 grams of body weight. The animals which received either adrenalin or insulin did not have access to food during a three hour post injection period. Glycogen concentration is expressed as the glucose equivalent in milligrams per 100 grams of tissue (wet weight).

Experiments were carried out at 24, 48, 72, 168 and 240 hours after section of the sciatic nerve. Thus some of the observations were made before

TABLE 1

Average values for the glycogen concentration of skeletal muscle at various times after denervation

HOURS AFTER DENERVATION	NUMBER OF ANIMALS	GLYCOGEN IN MILLIGRAMS PER 100 GRAMS OF MUSCLE			
		Control muscle	Standard deviation	Denervated muscle	Standard deviation
24	9	669	60	664	56
48	9	615	115	565	114
72	5	647	88	414	4
168	5	670	26	350	51
240	5	608	51	295	9

and some after the peripheral ends of the cut nerve fibers had lost their viability. This procedure allowed a discrimination to be made between effects due to a loss of reflex pathways and effects associated with the atrophic process. The latter condition first becomes manifest in muscle at the time of the loss of nerve viability and occurs in the rat at from 48 to 72 hours after nerve section (Hines and Knowlton, 1933).

RESULTS AND DISCUSSION. No significant change occurred in the glycogen concentration of a muscle during the first 24 hours following nerve section (table 1). A slight but significant decrease was noted after 48 hours. The denervated muscles suffered the greatest loss in their glycogen stores during the 24 hour period between 48 and 72 hours after nerve section. Of the total reduction in glycogen stores that occurred during the first 10 days after denervation more than 50 per cent was lost during the third day following nerve section. Further reductions in the concentration of muscle glycogen were found to be relatively small. The onset of the rapid decline in muscle glycogen stores coincided in time with the loss

of irritability in the cut nerve and the appearance of a ponderable weight loss and fibrillary contractions in the denervated muscle. Because of the time following denervation at which changes were first noted, we believe that the decreases in the glycogen stores are not due to a loss of reflexes directly concerned with glycogen synthesis and breakdown but rather to a peculiar condition of the atrophic muscle which is initiated at the time of the collapse of the viability of the neural elements.

The experiments (table 2) carried out 24 hours after denervation indicate that such muscle was as efficient as innervated tissue in storing ingested sugar as glycogen. Further evidence of a normal capacity to store glyco-

TABLE 2

Average values for the glycogen concentration of control and denervated muscle 3 hours after the oral administration of 0.75 gram of glucose per 100 grams of body weight

EXPERIMENTAL CONDITION	HOURS AFTER DENERVATION	GLYCOGEN IN MILLIGRAMS PER 100 GRAMS OF MUSCLE			
		Control muscle		Denervated muscle	
		Without glucose	After glucose	Without glucose	After glucose
Non-fast	24	669	720	664	702
96 hour fast*	24	380	860	380	809
Non-fast	48	615	711	565	546
Non-fast	72	647	740	414	378
Insulin†	72	647	798	414	411
48 hour fast	72	478	759	394	415
Insulin‡	72	421	784	122	373
Adrenalin‡	72	391	795	196	434
Non-fast	168	670	702	350	322
Adrenalin‡	168	425	847	107	458

* Fasted prior to denervation.

† One-fourth unit insulin administered at time of glucose.

‡ Administered 3 hours before glucose.

gen during this time was found in the experiments in which the muscle glycogen was greatly reduced by fasting prior to the time of denervation (table 2). The presence of a nerve supply was not essential for the restoration of glycogen stores which had been lost during the previous period of fasting. This apparently unaltered capacity of denervated muscle to store glycogen during the first 24 hour period stands in marked contrast to the findings in the later periods in which the ingestion of sugar failed to raise the glycogen concentration in muscle which had been denervated for periods of 48, 72 and 168 hours. This failure was also observed when the glucose was accompanied by small amounts of insulin. It is thus apparent that for some time, 24 hours or longer, the muscle deprived of its nerve

supply was able to maintain normal glycogen stores and to increase them above the characteristic level in response to glucose ingestion, in a manner paralleling the behavior of normal control muscle. This state of apparent normalcy was, however, of short duration because at about the second day after denervation an abrupt change occurred in the capacity of such tissue to synthesize and store glycogen. Further evidence was sought as to the cause of the breakdown of glycogen storage in denervated muscle. A comparison was made of the efficiency of control and denervated muscle to rebuild the losses of glycogen stores brought about by the administration of adrenalin and insulin in amounts which had been found in other experi-

TABLE 3

The influence of adrenalin, insulin and thyroxin administration and fasting upon the glycogen concentration of control and denervated muscle

EXPERIMENTAL CONDITIONS	HOURS AFTER DENERVATION	NUMBER OF ANIMALS	GLYCOGEN IN MILLIGRAMS PER 100 GRAMS OF MUSCLE			
			Control innervated muscle	Amount of decrease	Denervated muscle	Amount of decrease
Control	72	5	647		414	
Adrenalin*	72	10	391	256	196	218
Insulin†	72	9	421	226	122	292
48 hour fast	72	8	478	169	394	20
Control	168	5	670		350	
Adrenalin*	168	6	425	245	107	243
Thyroxin	168	10	659	11	224	126
96 hour fast	168	13	380	290	235	115

* Tissues removed 3 hours after subcutaneous injection of 0.02 mgm. of adrenalin per 100 grams body weight.

† Tissues removed 3 hours after subcutaneous injection of 1 unit of insulin per 100 grams body weight.

ments (table 3) to be sufficient to cause a marked reduction in the glycogen concentration of both tissues. The results (table 2) indicate that the denervated muscle was quite efficient in restoring the glycogen concentration up to its characteristic level. It would appear as if the muscle at this time had not lost its power of glycogen synthesis but rather that the conditions in the muscle were not favorable for the synthesis and storage of glycogen above a certain concentration. Why this condition which might be called a "saturation point" exists in muscle under these conditions is not clear. Further evidence must be sought concerning the distribution of glycogen in the cellular structures under such conditions and as to whether denervation is followed by a collapse of glycogen storage in certain cellular structures and not in others.

The results of the experiments (table 3) in which adrenalin and insulin were administered indicate that a denervated muscle suffered the loss of as much glycogen as its control. The per cent of glycogen lost was greater in the former than in the latter. It is evident that these glycogenolytic agents were effective upon muscle in the absence of a nerve supply. The results in some instances suggest an increased sensitivity of the denervated tissue to these hormones. However, the glycogen concentration in a tissue represents a balance between synthesis and breakdown. The ability of a denervated muscle to synthesize glycogen in amounts up to its characteristic level has been demonstrated but it is not apparent whether the intensity of this process is subnormal or not. It is possible that this apparent increased sensitivity to glycogenolytic agents is due to decreased glycogen synthesis rather than to increased glycogenolysis.

Thyroxin administration resulted in a lowering of the glycogen concentration in the denervated muscles and no change in that of the control muscles (table 3). Apparently much larger doses of thyroid hormone or longer periods of time for its action are required in order to lower appreciably the glycogen stores of normal muscle. It would appear as if the denervated muscle behaved more like cardiac muscle in its sensitivity to the thyroid hormone.

Fasting was effective in reducing the glycogen stores of denervated muscle (table 2). This observation is not in accord with the findings of Wertheimer (1927) as to the effects of fasting upon the glycogen stores in denervated muscles of cats, dogs, guinea pigs and rabbits. However, the effects of fasting may be masked in the early periods after operation by the concomitant decrease in glycogen stores due to the effects of denervation per se. The question may be raised as to whether fasting and thyroxin reduced the glycogen stores indirectly by accelerating the rates of atrophic changes or by a more direct effect upon the tissue. It would appear as if the lowering of muscle glycogen in both conditions was greater than that observed in control animals with equivalent amounts of weight loss from the muscles.

We believe that the above experiments demonstrate that glycogenic and glycogenolytic reflexes play little or no part in the regulation of glycogen synthesis and breakdown in the skeletal muscle of the rat. That stimuli from the nervous system may induce contractility and thereby alter glycogen concentration in the muscle is, of course, granted. The finding of a normal glycogen content and an unaltered capacity to synthesize and store glycogen in muscle for some time after denervation is evidence against the existence of important glycogenic reflexes. Our experiments demonstrate that only after a certain period of time following denervation, do profound changes occur in the glycogen content and capacity to synthesize and store additional amounts of this material. It is interesting to note that the

onset of this altered condition in the muscle coincides in time with beginning of ponderable weight loss from such tissue and the exhibition of altered physiological properties which are characteristic of denervation atrophy. It appears that the time at which the cut nerve loses its excitability marks the appearance of an altered functional state in the muscle. As far as can be determined, a normal physiological state persists in the muscle until these neural elements lose their viability. Thereupon occurs a collapse or exhaustion of something in the neuromuscular relationship which is immediately followed by marked changes in the composition and physiological characteristics of the muscle.

It cannot be determined from these experiments whether the changes in glycogen metabolism are due primarily to autonomic or somatic denervation. Some investigators (Herrin and Meek, 1931; Britton, 1930) observed that mammalian skeletal muscle suffered a diminution in glycogen content following the loss of its sympathetic nerve supply. Other workers (Dworkin, Bacq and Dill, 1931; Beattie, Beattie and Milroy, 1930) have found no marked or consistent effect of sympathectomy upon muscle glycogen. Moreover the loss of the sympathetic nerve supply does not result in atrophic changes in the muscle comparable to those following somatic denervation. The finding that the earliest detectable signs of an altered glycogen metabolism are concomitant with the occurrence of other changes peculiar to denervation atrophy suggests that the changes reported here are more closely related to the loss of the somatic than of the autonomic innervation.

The results of the experiments described herein are not in accord in many respects with the findings of other investigators as to the effects of denervation upon the glycogen metabolism of skeletal muscle. It is believed that, in some instances, this can be attributed to a species difference. This explanation finds support in the observation by Wertheimer (1927) that glucose ingestion in the dog and cat resulted in little or no increase in the glycogen concentration of denervated skeletal muscle which had previously been reduced by fasting and phloridzin; while an unhampered capacity of such muscle to form glycogen was noted in similar experiments upon guinea pigs and rabbits. However, it is apparent that in many experiments the condition of the animal and the techniques employed for the removal of tissue for analysis have been such as would allow considerable change to occur in the glycogen content of the muscle. It is conceivable that some of the procedures employed, such as killing the animals by stunning and hemorrhage, would not have an equal effect upon the glycogen stores in normal and denervated muscle. Furthermore, the necessity of giving proper attention to the time after denervation at which studies are made is apparent from our experiments.

SUMMARY

A study has been made concerning the glycogen metabolism of denervated skeletal muscle. The experiments were carried out on the gastrocnemius muscle of the rat. The corresponding muscle of the opposite unoperated limb served as a control.

The glycogen content of the denervated muscle was the same as that of its control for, approximately, 48 hours after section of the sciatic nerve. A marked decrease in the glycogen concentration occurred during the third day following denervation. Further decreases were noted in the experiments involving longer periods of denervation but these changes were relatively gradual and small.

Glucose administration was accompanied by normal glycogen storage in the denervated tissue of well fed animals at 24 hours after operation; but no storage could be demonstrated when the experiments were carried out two days or longer after nerve section. However, when the glycogen stores of these tissues had been previously depleted by the administration of either adrenalin or insulin, it was found that glucose ingestion was accompanied by similar glycogen deposition up to but not above the concentration existing in muscles prior to the action of these glycogenolytic agents.

The administration of adrenalin and insulin resulted in approximately equal decreases in the glycogen content of control and denervated muscle. Thyroxin in amounts insufficient to reduce the glycogen content of normal muscle caused a lowering of glycogen concentration in denervated muscle. Fasting caused a reduction in the glycogen stores of denervated muscle. This effect of fasting may be masked in the early periods after operation by the concomitant decrease in the glycogen stores due to denervation.

The finding of a normal glycogen content and storage capacity in muscle for a period of 24 hours after denervation is regarded as evidence against the existence of important reflexes concerned directly with glycogen metabolism. The time after denervation at which muscle suffers a marked decrease in its glycogen concentration and ability to store ingested sugar as glycogen above a certain characteristic level corresponds in time to the loss of viability of the cut nerve and the appearance of altered physiological properties of the muscle, peculiar to denervation atrophy.

REFERENCES

BEATTIE, F. J. R., M. K. BEATTIE AND T. H. MILROY. J. Physiol. **69**: 364, 1930.
BRITTON, S. W. This Journal **93**: 213, 1930.
DWORKIN, S., Z. M. BACQ AND D. B. DILL. This Journal **96**: 308, 1931.
HERRIN, R. C. AND W. J. MEEK. This Journal **97**: 57, 1931.
HINES, H. M. AND G. C. KNOWLTON. This Journal **104**: 379, 1933.
KNOWLTON, G. C. AND H. M. HINES. This Journal **109**: 200, 1934.
MACLEOD, J. J. R. Bull. Johns Hopkins Hosp. **54**: 79, 1934.
WERTHEIMER, E. Pflüger's Arch. **215**: 779, 796, 1927.

REACTIVITY OF THE UTERUS TO PRE-SACRAL NERVE STIMULATION AND TO EPINEPHRINE, PITUITRIN AND PILOCARPINE ADMINISTRATION DURING CERTAIN SEXUAL STATES IN THE ANESTHETIZED RABBIT[1]

JOHN J. SAUER, CECELIA E. JETT-JACKSON AND SAMUEL R. M. REYNOLDS

From the Department of Physiology, Long Island College of Medicine, Brooklyn, N. Y.

Received for publication December 4, 1934

Despite the existence of an abundance of data relating both to the effect of sympathetic nerve stimulation upon the uterus and to the effect of pituitrin, epinephrine and pilocarpine upon the uterus, nevertheless an adequate correlation of the effects of these several agents with certain sexual states is still lacking. Many observations have been made on the reactivity of the excised pregnant uterus in a variety of animals, as have similar observations on the oestrous uterus of the rat, rabbit, guinea pig, cow, human and other forms (for review, see E. Allen, 1932, and Robson, 1934).

Information from uteri *in situ* scarcely exists, however, except for relatively few observations of van Dyke and Gustavson, 1929; Pompen, 1933; Rudolph and Ivy, 1930, and others in the rat, rabbit, cat, dog and monkey. Even so, such reports usually distinguish the sexual condition of the animal merely as "pregnant" and "non-pregnant" or as "virgin" and "multiparous." Obviously, with our present knowledge of reproductive phenomena, and of the hormones which control them, such distinctions are unsatisfactory.

Accordingly, the present work was undertaken to carry out a systematic investigation of the effects upon the uterus of presacral nerve stimulation, and epinephrine, pituitrin and pilocarpine administration in castrated rabbits, some of which were injected with oestrin, and some not; in rabbits at selected periods of pseudopregnancy; and finally in rabbits whose uteri were subjected to prolonged luteal activity. The use of animals in these sexual states renders it possible to ascertain the nature of the uterine responses 1, in the absence of ovarian hormonal influence; 2, with specific replacement therapy of the pure hormone oestrin, alone, and 3, with the

[1] Aided by a grant from the Committee for Problems in Research of Sex, of the National Research Council.

hormonal influences associated with the formation, full activity and waning effect of the corpus luteum.

METHODS. Twenty-three virgin rabbits weighing between 1.6 and 3.6 kilograms were used in the following experiments.

As a preliminary treatment, eight of these were castrated under aseptic conditions, and four used as castrates, while four others received oestrin (Theelin, Parke, Davis & Co., intravenously in 50 r.u. amounts and Amniotin, E. R. Squibb & Son, subcutaneously in 100 to 200 r.u. amounts) for two to four days preceding the experiments. In another group of experiments eleven rabbits were injected with human urine of pregnancy. Some were used in experiments on the first and second, others on the sixth and seventh, and the remainder on the eleventh and twelfth days of the resulting pseudopregnancy. Four other rabbits were injected with urine of pregnancy twice, the second time on the eleventh day of pseudopregnancy and were used on the eighteenth day after the first injection. As a result of such treatment, these rabbits were maintained in a state of continued luteal activity for about a week longer than would have been the case if only the one injection had been employed.

Dial (Ciba) anesthesia in dosages of 0.75 cc. per kilogram of body-weight was injected intramuscularly, one and a half to two hours before an experiment. Ether was used in minimal amounts to supplement the early action of dial until the operation of exposing the uterus was completed. At all times the animals were kept under as light anesthesia as possible.

In exposing the uterus, a long mid-line abdominal incision was made. The abdominal viscera were retracted and permanently held aside with gauze to give free access to the ovaries, uteri and aorta. When necessary, the bladder was retracted too. The middle of the left horn of the uterus was held in two places by silk sutures to the reflected left side of the abdominal wall. Mid-way between these two points another thread was passed through the outer uterine muscle wall, tied, and a free end was attached to a long light lever over a pulley. The lever was balanced and under just enough tension to be horizontal. The pre-sacral nerve (Davis, 1933) was found on the anterior surface of the aorta near the bifurcation, at the level of the third or fourth lumbar vertebra. The uterus and bladder were covered with dry absorbent cotton and the animal was covered with towels, care being taken that the covering in no way interfered with the thread running from the uterus to the lever. The average time for the operation was twenty-two minutes. An additional interval was allowed to elapse so that half an hour to one and a half hour intervened between completion of the operation and commencement of the observations.

Tetanizing currents lasting for one to two minutes served as the source of electrical stimulation. In those instances in which a positive response, or a contraction of the uterus, resulted, the uterus blanched and the colon became markedly contracted.

The dosages of the various drugs, injected in the marginal ear vein, were epinephrine, 0.1–0.2 cc. of 1:10,000 dilution; pilocarpine, 1 cc. containing 1/100 grain; Pituitrin-S (Parke, Davis & Co.), 0.1 cc. Fifteen to sixty minutes were allowed to elapse between injections.

RESULTS. The results, briefly discussed below, are summarized in table 1.

Oestrin-injected rabbits. In only one of the four sexual conditions studied, as outlined above, were positive responses elicited by each of the stimulating agents employed. Thus only in the oestrin-injected castrates

were sympathetic nerve stimulation and injection of epinephrine, pituitrin, or pilocarpine equally effective in eliciting contractions of the uterus with this method of study. Moreover, as shown in table 1, the pilocarpine response may be inhibited if it is preceded by atropine. When one investigates the action of these same chemical agents on uterine fistulae in unanesthetized rabbits, it also is found that they are equally effective (Reynolds, 1930, 1933; Blair-Bell, Datnow, and Jeffcoate, 1933; Pompen, 1933).

Castration. In the castrated rabbit, and so in the absence of ovarian hormones, one finds that strong contractions are elicitable by each of the foregoing stimulating agents, with the single and invariable exception of pilocarpine. This drug failed to induce any detectable response in the castrated rabbit. The positive effects of pituitrin and epinephrine in castrates contrasts with the findings that have been obtained in studies

TABLE 1

	CASTRATES 17–37 DAYS	CASTRATES + OESTRIN	PSEUDOPREGNANCY			PROLONGED LUTEAL ACTIVITY—18 DAYS*
			1–2 days	6–7 days	11–12 days	
Pre-sacral nerve stimulation	+	+	0	0	+	0
Epinephrine	+	+	(+)	+	+	+
Pituitrin	+	+	+	+	+	+
Pilocarpine	0	+	0	0	0 and (+)	0
Pilocarpine after atropine		−			−	

(+) signifies that the responses obtained were feeble.
*Many typical corpora lutea, slight endometrial proliferation.

with the uterine fistula, for with this technic in the unanesthetized rabbit these drugs are without effect. It is important to note, therefore, that under the conditions of the present experiments the atrophied uteri of castrates respond to these two drugs. As a consequence, the failure of pilocarpine to elicit a contraction in the present experiments affords the sole distinguishing criterion as to whether or not oestrin is acting on the uterus,—a distinction that may not be made with the fistula technic since all three drugs are equally ineffective in the castrate (Reynolds, 1933; Blair-Bell et al., 1933).

Pseudopregnancy. In the third sexual condition studied, namely, that of pseudopregnancy, several peculiar differences are noted in the uterine responses when they are compared to those described above. For instance, within the first two days of pseudopregnancy, a reversal of the response to pre-sacral nerve stimulation occurs and feeble responses only

result from the injection of the relatively large dosage of epinephrine used in these experiments. No reversal to the action of pituitrin was found in this series, but the contractions obtained were in no case of long duration, such as one finds with the uterine fistula.

At the end of a week of pseudopregnancy, as shown in table 1, when the corpus luteum is at the height of its function as judged by the extent of endometrial proliferation, the responses obtained, except for a slightly greater effect of epinephrine, are identical with those found in early pseudopregnancy. Two of three rabbits used at this time showed strong contractions following the usual amount of epinephrine. It is clear, therefore, that an inhibited response to nerve stimulation is the only response regularly modified by the hormonal influence of the corpus luteum. This differs from findings of van Dyke and Gustavson in the cat, in which animal the effect of epinephrine faithfully parallels the nerve responses.

Later in pseudopregnancy, on the eleventh and twelfth days, maximal nerve responses are once again obtained: the "pregnancy-reversal" is itself reversed. Two of the four rabbits in this series gave feeble, yet nonetheless definite, responses following pilocarpine administration. In view of the ineffectiveness of this drug at any time other than when oestrin alone is acting upon the uterus, these results suggest that the rabbits showing the feeble pilocarpine responses were commencing to elaborate oestrin. In rabbits with uterine fistulae at this stage of pseudopregnancy, the uterus is invariably quiescent and refractory to pituitrin as well, so the present data do not parallel those in which the balloon method in the unanesthetized animal is employed.

Prolonged luteal activity. A state of prolonged luteal activity, as in these experiments, is wholly artificial for the rabbit in the absence of gestation. It was investigated in the present work, however, because of the fact that it has been reported recently (Leonard, Hisaw and Fevold, 1932) that potent corpus luteum extracts cannot maintain the proliferated state for long. McPhail (1933) reports that a second set of corpora lutea will form following a second injection of human pregnancy urine, however, and these corpora later will maintain the endometrium in a proliferated state. This has not been our experience, for although there were many corpora lutea at the time of the experiments, on the eighteenth day even so the extent of endometrial proliferation at this time is inappreciable. This has also been the experience of W. M. Allen (personal communication). Since in the present work the irritability of uterine muscle to nerve excitation is found to be profoundly modified by the presence of corpora lutea, as indicated above, it was possible in this series of experiments to test the functional capacity of the second crop of corpora lutea.

As indicated in table 1, the uterus showed no response to pre-sacral nerve stimulation. Thus the pregnancy-reversal persists after eighteen

days of luteal activity *and in the absence of luteal effect on the endometrium.* It is evident, therefore, that the corpora lutea are functional and that a dissociation of the two luteal effects, endometrial proliferation and uterine muscle inhibition, is demonstrated by these results. A similar dissociation of these two effects has also been observed in rabbits with uterine fistulae, in which it was observed that large amounts of oestrin prevented the usual proliferating action of the rabbits' own corpora lutea but the usual motility-stimulating action of oestrin was held in abeyance by the progestin elaborated by the corpora lutea (Reynolds and Allen, 1932; Allen and Reynolds, in press).

Discussion. Our positive results with pituitrin vary from those reported for excised uteri removed during pseudopregnancy. The reason for the discrepancies between the present and other technics is not clear, although it is possible that the experiments described above may be explained in the light of Pompen's observations through an abdominal window in unanesthetized rabbits. This investigator notes that not only is the threshold for pituitrin responses much higher in castration and during pseudopregnancy than when the uterus is under the influence of oestrin, but the contraction appears to be weaker and less of a spasmodic type in which the whole uterus contracts simultaneously (Pompen, 1933). Since in the present work the recorded contraction represents activity of a relatively isolated spot on the surface of the uterus, it is likely that the feeble contractions which Pompen notes are well recorded in our experiments.

The fact that the pituitrin and epinephrine responses described above do not agree with what one might expect on the basis of *in vitro* and other *in vivo* observations, raises at once a question as to what extent one may make physiological inferences from data with this method of investigation. It may be argued that the operative procedures, the anesthetic or some other abnormal experimental condition modifies the normal physiological activity of the uterus. This may not be denied, and it only serves to emphasize that the physiological behaviour of any organ must ultimately stand the test of scrutiny in the body under as nearly normal conditions as possible, preferably in the non-anesthetized animal.

For the purposes of the present problem, however, the present data have proven to be satisfactory. It is possible to state, for instance, that under the conditions of these experiments, a pilocarpine response depends upon an oestrin-activated uterus, and that the action of the corpus luteum is to reverse the response to sympathetic nerve excitation, in contrast to the contraction that ensues after such stimulation in an oestrin-injected or castrated rabbit. Thus, the *reactivity* of the uterus to these two agents under the conditions of these experiments provides a basis for estimating the hormonal influence of the ovary at the time, provided other complicating conditions do not interfere.

In conclusion, a word may be said concerning the reversal of the uterine response due to the action of the corpus luteum. During the oestrin phase of ovarian activity in the cat, the uterus relaxes following sympathetic nerve excitation, whereas in the rabbit, the uterus contracts. On the other hand in the presence of a corpus luteum the uterus of the cat contracts (van Dyke and Gustavson) while in the rabbit, as mentioned above, no contraction ensues upon excitation of the pre-sacral nerve. In two instances, in fact, the records show that the uteri relaxed. Perhaps inadequacy of our technic prevented records of relaxation in the remaining rabbits of this series. Suffice it to say, however, that the presence of a corpus luteum in either the cat or rabbit effects a reversal of the pre-pregnancy or pre-pseudopregnancy state, so that both animals show a "pregnancy-reversal" in the sense intended by van Dyke and Gustavson.

SUMMARY

1. Pilocarpine elicited a contraction only when the uterus was under the influence of oestrin. (Failure to do so in castrated and pseudopregnant rabbits.)

2. Uteri of rabbits castrated for 17 to 37 days contracted in response to pre-sacral nerve excitation, epinephrine, and pituitrin administration (in spite of markedly atrophied condition).

3. When corpora lutea were present, the uterus did not contract in response to nerve excitation ("pregnancy-reversal"). A reversal of response to epinephrine did not occur.

4. In pseudopregnancy the corpus luteum functioned, as regards inhibition of uterine reactivity, on the second, and the sixth and seventh days. By the eleventh day its influence had waned.

5. Under the influence of the corpus luteum for 18 days the endometrium failed to be maintained in a proliferated condition, yet the uterus was refractory to pre-sacral nerve stimulation. The corpora lutea were functional, therefore, and the dissociation of the effects upon the endometrium and muscle reactivity respectively was due not to a deficiency or inadequacy of the lutein hormone, progestin, but rather to the inability of the endometrium to remain proliferated in the face of what is normally the adequate and specific stimulus for endometrial proliferation.

It is a pleasure to acknowledge that our interest in a closer correlation between the reactivity of the uterus and specific phases of sex hormonal influence was aroused by a conversation with Doctor Robert T. Frank and one of us (S.R.M.R.).

REFERENCES

ALLEN, E. Sex and internal secretions. Baltimore, 1932.
ALLEN, W. M. AND S. R. M. REYNOLDS. In press.

BLAIR-BELL, W., M. DATNOW AND T. N. A. JEFFCOATE. J. Obst. Gynec. Brit. Emp. **40**: 541, 1933.
DAVIS, A. A. J. Obst. Gynec. Brit. Emp. **40**: 481, 1933.
LEONARD, S. L., F. L. HISAW AND F. H. FEVOLD. This Journal **100**: 111, 1932.
MCPHAIL, M. K. J. Physiol. **79**: 118, 1933.
POMPEN, A. W. M. Thesis, Amsterdam, 1932.
REYNOLDS, S. R. M. This Journal **92**: 430, 1930.
Proc. Soc. Exp. Biol. Med. **30**: 1167, 1933.
REYNOLDS, S. R. M. AND W. M. ALLEN. This Journal **102**: 39, 1932.
ROBSON, J. M. Recent advances in physiology of sex and reproduction. 1934.
RUDOLPH, L. AND A. C. IVY. Am. J. Obst. Gynec. **19**: 317, 1930.
VAN DYKE, H. B. AND R. G. GUSTAVSON. J. Pharmacol. Exp. Therap. **37**: 379, 1929.

THE FUNCTION OF THE BRAIN IN OLFACTION

The Effects of Large Cortical Lesions on Olfactory Discrimination[1]

H. G. SWANN

From the Departments of Physiology and Psychology, University of Chicago

Received for publication November 23, 1934

In a previous article (1933), the author reported an apparatus upon which rats readily learn to discriminate between two odors. It consists, briefly, of two interchangeable entrance boxes to a food compartment, each of which is blocked with differently scented wood shavings. In order to get to the food, the rat digs through the shavings and then noses up a trapdoor. The trapdoor of one of the boxes is shut so that the rat cannot enter, but has to choose the box having the proper odor in its shavings. A trained animal only noses at the shavings in the wrong box and then proceeds to the other and correct box. A rat is considered to have learned the discrimination when it performs 27 times correctly out of 30 consecutive trials; using this criterion, learning is rapid, some 120 animals averaging about 80 trials to learn. Controls of all other senses indicated that olfaction only is involved in the discrimination (1933).

The author also determined (1934) the effects of lesions to the central nervous system on this learned habit, in an attempt to find which nervous structures were essential to its performance. It was found that lesions to the olfactory bulbs were followed by a reduction in ability to perform the discrimination, complete if the bulbs were excised. Interruption of the lateral and medial olfactory tracts did not interfere with the discrimination, but lesions to the anterior limb of the anterior commissure caused a distinct loss in ability. Lesions to structures in the rat's olfactory nervous system higher than these, including septum, fimbria, fornix, amygdala, hippocampus, and pyriform lobes, had no effect on the discrimination. The entire neopallium also was explored with similarly negative results. Such data indicate that we are dealing with olfaction, but it is difficult to account for the fact that no cortical structure was found necessary to the performance of this learned olfactory discrimination. This method and these data fail entirely to confirm the neuroanatomists' belief that about two-thirds of the rat's cortex is olfactory in function.

[1] It is a pleasure to acknowledge the aid and advice of Dr. K. S. Lashley during this work.

To explain these results, the author suggested either sub-cortical learning or else a dynamic organization of the cortex similar to that found by Lashley (1929) in maze performance, i.e., all of the brain being involved in the performance of the habit, with parts other than those excised being readily able to take over the function. The present paper reports the test of these theories: if a dynamic organization were involved, there should be found a limiting percentage of cortex beyond which performance would be impossible. If on the other hand retention were sub-cortical, the animal should perform irrespective of the amount of pallium functional. The effect of large lesions was, accordingly, to be determined.

TABLE 1

NUMBER	POSITIVE ODOR	TRIALS			PER CENT CORTICAL DESTRUCTION	DAYS BEFORE PERFORMANCE
		Learning	Pre-operative retest	Post-operative retest		
46	A	130	20	0	32.8	11
47	A	0	0	0	34.1	3
48	D	30	10	0	35.8	0
49	D	90	0	0	36.0	4
50	A	0	0	0	57.9	10
51	D	40	10	0	58.3	7
52	D	30	0	0	58.8	15
53	A	40	0	0	66.0	13
54	A	0	0	0	66.2	13
55	D	50	0	10	85.2	11

Performance records of all cases. "A" and "D" show to which of the two odors, anise or disinfectant, the animal was positive; it was negative to the other odor of the pair. Under "Trials," each figure presents trials exclusive of those made while attaining the criterion of 27 correct responses out of 30 consecutive trials. Thus the figure "0" shows that the rat reached the criterion within the first 30 trials. The figures under "Days before performance" are explained in the text.

METHODS. Ten rats were trained, each being given 10 trials a day, to discriminate between the odors of anise and "Sheep Dip and Disinfectant" (odor of creosote). After learning, they were given 14 days' rest and were then retested; this was done in order to find the influence of lapse of time alone on the discrimination. Next, under ether anesthesia, a large lesion to the rat's brain was made with an electric cautery. In some cases, a two-stage operation was made. Fourteen days' recuperation was allowed and finally the ability to discriminate retested. The brains were sectioned and stained, and the extent and locus were determined by histological examination and projection upon Lashley's diagram (1929). The relative area of the lesion was computed after measurement with a planimeter.

RESULTS. In table 1 are listed the learning and retest records, and the per cent of cortex excised in each animal. In figure 1 each rat's lesion is

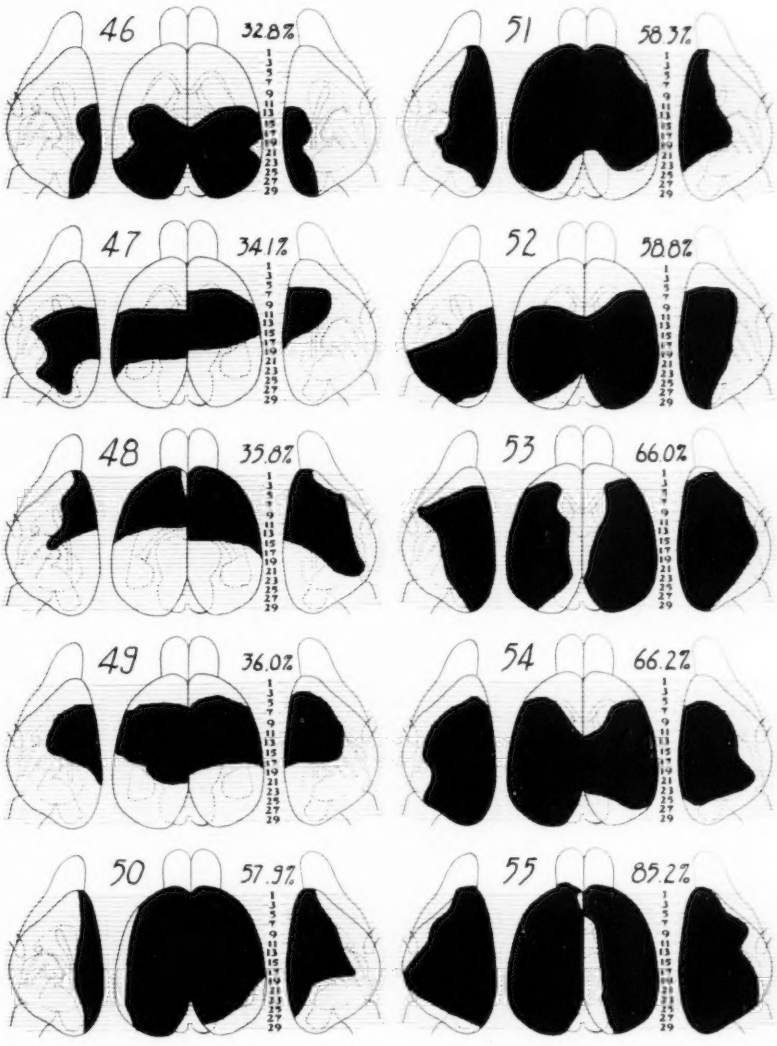


Fig. 1. Diagrams of the extent and locus of lesion in individual cases. Cases are numbered to correspond with those described in the text. The percentage destruction of the cortex is also shown for each case.

shown. From these data it will be seen that excisions up to 85 per cent of the cortex do not produce any interference in the discrimination. The slight loss in the case of number 55 will be discussed below.

The number of days that the operated animal spent on the apparatus before actually commencing performance was included because of the pro-

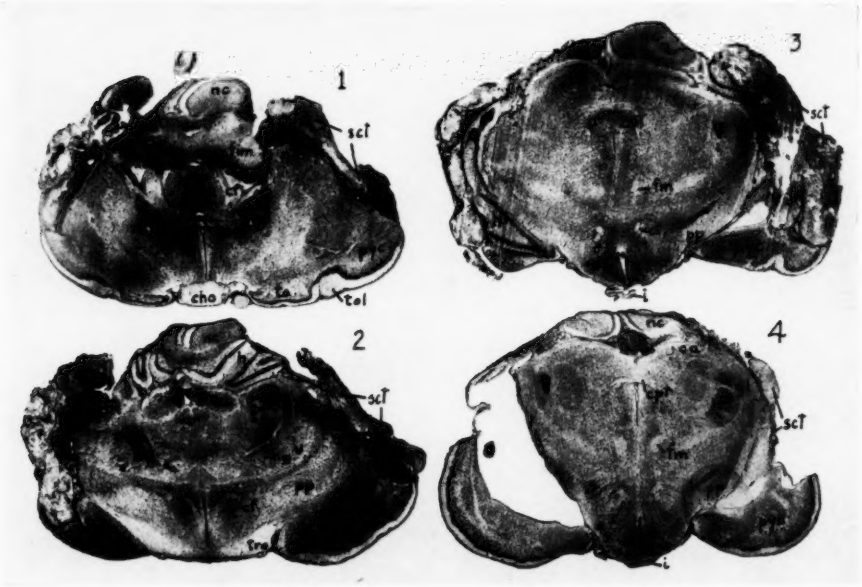


Fig. 2. Numbers 1, 2 and 3, are photomicrographs of the brain of case number 55, being transverse sections at levels 12, 16 and 22 respectively of the projection of this same brain in figure 1. The cortex has been largely excised, only scar tissue remaining.

Number 4 shows a transverse section of the brain of number 53 at level 21. The hippocampus has been clearly excised. All sections × 6, thionin stain.

Key to lettering in figure 2: *ca*, anterior colliculus; *can*, anterior commissure; *cau*, caudate nucleus; *cc*, corpus callosum; *cf*, columns of fornix; *cho*, optic chiasma; *cpt*, posterior commissure; *fim*, fimbria; *fm*, fasciculus retroflexus; *gl*, lateral geniculate body; *h*, hippocampus; *hb*, habenula; *i*, infundibulum; *nc*, neocortex; *pp*, basis pedunculi; *pyc*, pyriform cortex; *s*, septum; *scf*, scar tissue; *to*, olfactory tubercle; *tol*, lateral olfactory tract; *tro* optic tract; *v*, ventral thalamic nucleus.

nounced diasehisis noted. It was, as table 1 shows, roughly proportional to the amount of cortex excised. In the case of these large cortical lesions, it was only after a long time that the rat performed the already learned

discrimination. Errors were not made, but the rats just did not attempt to enter either box. Behavior typical to large brain lesions was obvious, viz., aimless running from side to side, pronounced motor difficulties, asthenia and ataxia. Thus: animals would constantly fall off the apparatus, would climb into one of the boxes and be unable to get out for a half hour, would get apparently stuck at the corner of a 90° plane surface and spend two minutes trying to go forward, and would, when placed in the food compartment, never notice the food. Such behavior occurred for a period, and then suddenly the animal would start to perform correctly. This abrupt change from apparently senseless behavior to motivated behavior was striking; we can best explain it on the assumption that some diaschitic effect had worn off.

In order to get the animals to start, some urging was resorted to. Also, because of the asthenia in some cases, both entrances were very lightly blocked with the scented shavings. In general, however, as noted above, once the animal could be gotten to start performance, it proceeded with great style.

The lesion of number 55 was the largest in this series; in it 85.2 per cent of the cortex was destroyed. The two cortical olfactory structures of greatest size are the hippocampus and pyriform lobes, yet in this case 89 per cent of the former and 78 per cent of the latter were excised without interfering with this olfactory habit. Performance of the discrimination of this rat, after 11 days of the sort of demented behavior described above, was clear; its score, in number of trials correct out of the daily ten, was 7, 9, 9, 10. Performance was not, as in the other cases, perfect, but the loss was so slight as to be negligible; furthermore, this sort of loss, as determined in the previous study (1934), is not exceptional.

Similarly, case 53, with 66 per cent of the entire cortex and 78 per cent of the hippocampus excised showed no reduction in efficiency. Photomicrographs of these two brains are shown in figure 2.

DISCUSSION AND SUMMARY

Lashley (1929) determined that destruction of any 25 per cent, approximately, of the rat's cortex produced great interference in maze performance. Since, however, our lesions run up to 85 per cent of the cortex without producing any interruption in this learned olfactory discrimination, it seems clear that the discrimination is not mediated by a dynamic equivalence of all parts of the rat's cortex. In the previous study (1934), the records of 45 cases indicated that no specific cortical structure or cytoarchitectural area was essential to the performance of this learned habit. While 100 per cent decortication was not accomplished, nevertheless, since removal of 85 per cent of the cortex produced no interference in this learned habit,

the assumption that this olfactory habit in the rat is subcortical appears well founded.

REFERENCES

LASHLEY. Brain mechanisms and intelligence. Chicago, 1929.
SWANN. J. Comp. Psychol. **15**: 229, 1933.
J. Comp. Neurol. **59**: 175, 1934.

NOTE ON REFLEX THRESHOLDS IN THE CAT DURING SPINAL SHOCK

G. P. McCOUCH, W. J. SNAPE AND W. B. STEWART

From the Department of Physiology and the Department of Neurology, University of Pennsylvania Medical School

Received for publication November 17, 1934

For the analysis of spinal reflexes the acute spinal cat is more frequently employed than any other animal. One reason for its use is its relative immunity from spinal shock. It is, therefore, of interest to determine the extent of this immunity, the degree of stability of such a preparation. Yet such a standardization has not supplied the incentive for the present work. The experiments reported here were designed primarily to elucidate a disputed point concerning the susceptibility of the flexion reflex to spinal shock.

The term "spinal shock" is used in the present paper to designate the transient depression of reflexes incident to spinal transection in segments below the level of severance. It does not refer to the permanent features differentiating the spinal animal from either the normal or the decerebrate. Hence it can be measured only in terms of reflex recovery in the spinal animal.

In 1910 Sherrington noted the fact that in the decerebrate cat the ipsilateral flexion reflex of the lower extremities is increased immediately subsequent to spinal transection (9), (10). This observation has led many physiologists (1), (2), (3) to question the occurrence of shock to this reflex in the cat. This conclusion could have been reached logically only by employing the term "spinal shock" in a less restricted sense than that defined above.

In 1898 Sherrington (8) established the relation between the development of descending pathways and the severity of the reflex depression induced by transection and also the marked difference in the degree to which different reflexes are affected. In the cat the phenomenon is slight and evanescent. Among the reflexes, ipsilateral flexion is one of those least involved. The only quantitative estimate of its involvement of which we are aware is that of Fulton and Sherrington (4). They compared acute and chronic spinal animals in regard to the tension of the maximal flexion reflex expressed as a percentage of that of the maximal neuromyal tetanus sampled isometrically in the tibialis anticus muscle. Of three cats, two

acute animals yielded 79 per cent and 80 per cent respectively; one chronic, 84 per cent. For their purpose of contrasting cat and dog with monkey the figures are impressive. For the decision between the alternatives of little and of no shock in the cat they are of less value due to the fact that the acute observations were made three to four hours after transection.

The slightly progressive enhancement in the flexion reflex in the cat immediately subsequent to severing the cord at the end of a relatively prolonged anesthesia with diethyl ether is a familiar observation. That part of the progressive change may be attributed to spinal shock is indicated by the fact that in the cat previously deprived of cortico-spinal innervation on one side, the motoneurons so deprived yield a flexion reflex immediately after transection greater than that of the other leg (7). This has been attributed to recovery prior to transection from the moiety of shock due to cortical deprivation. Owing to such recovery between operations, it is impossible to obtain an adequate picture of spinal shock in a cat decerebrated a sufficient time before section of the cord to eliminate the possibility of reflex depression from prolonged anesthesia with diethyl ether. Indeed, anesthesia of sufficient depth to eliminate descending impulses constitutes a block of the cord. Hence, if spinal shock be due to loss of such impulses, the moment of transection so far as concerns its effect upon shock is the moment when anesthesia attains this critical depth rather than that of anatomical severance of the cord (8). For these reasons a quantitative study of spinal shock in the cat requires a minimal period under an anesthetic from which recovery is virtually instantaneous. Such an anesthetic is divinyl ether (5).[1]

METHOD. In thirteen adult cats the threshold for the flexion reflex was followed after transection at or about the twelfth thoracic segment. Of these, three were transected aseptically and employed solely in the study of the more gradual alterations. In the ten acute experiments the attempt was made to follow the early changes from the moment of transection. In the first four animals transection was preceded by decerebration by the trephine method under diethyl ether anesthesia. In four others operation was limited to laminectomy and transection of the cord under divinyl ether. In two cats under divinyl ether Sherrington electrodes were applied to the internal saphenous nerve. Of these one was decerebrated one minute before transection. In all other experiments stimulation was applied through three stigmatic gilt electrodes, one in the pad of the second digit, one on the external surface of the leg midway between knee and ankle, and one on the external aspect of the thigh midway between hip and knee. In several instances a fourth electrode was placed on the forepaw. A large indifferent electrode in the form of a brass rod was inserted in the rectum.

[1] We are indebted to the courtesy of Merck and Company for the divinyl ether used in this research.

The threshold contraction was determined visually without a mechanical record. The stimuli employed were single break induction shocks and repetitive alternating charge and discharge of a condenser.[2] The two capacities used were 1 m.f. and 0.01 m.f. Repetitive induction shocks were employed in a few experiments, including both make and break shocks to diminish polarization.

In all but the earliest experiments frequent measurements of resistance were made. Since the initial purpose of these was merely to test stability as to depth of the stigmatic electrodes, and since a rapid method was required to provide readings between frequent determinations of threshold, it was not deemed necessary to avoid polarization. A constant current was used and peak readings noted on a microammeter. In several of the later experiments the Kohlrausch method was also employed with a thousand cycle oscillator.

Discussion of methods. The advantages of the stigmatic electrode are the privilege of selection of any desired spot upon the receptive field, that of stimulating receptors at various intensities with relatively slight spatial summation, and that of following the entire course of recovery. It had been hoped that a simple correlation might be found between resistance and threshold. This was not the case. In figure 1 are plotted thresholds of a spinal cat in a steady state. The depth of the thigh electrode was not altered and yielded a constant threshold. The paw electrode was inserted progressively deeper. When it was in the horny layer, both resistance and threshold were high; at an intermediate depth resistance was intermediate, the threshold to condenser charges and discharges was lowest; at a still greater depth, the resistance was low, but the threshold for the condenser had risen significantly. The curve for single induction shocks, on the other hand, shows its lowest threshold where the resistance is lowest. In the case of the condenser the amount of current is independent of the resistance, which alters only the intensity and duration. Here the threshold appears to be more dependent upon current density at the level of the receptors than upon the resistance of the circuit.

To investigate the class of receptors stimulated an experiment was run on one of us (W. J. S.) as subject. Weak single condenser charge or discharge and weak single induction shocks were reported as touch, strong ones as pain. Weak repetitive stimulation from the condenser gave tickle; slightly stronger stimulation, itch with appreciable after-discharge; still stronger, pain, increasing with increasing duration of stimulation, and greater after-discharge. To our disappointment, there was no difference in types of sensation induced by a hundred-fold change in condenser capacity, though the threshold for 0.01 m.f. was about three times the voltage

[2] We are indebted for the construction of this apparatus to Dr. Emil Bozler and to Mr. A. J. Rawson of the Eldridge Reeves Johnson Foundation for Medical Physics.

of that for 1 m.f. The same ratio obtained between reflex thresholds in the spinal animals.

RESULTS. *The ipsilateral flexion reflex.* From the time of transection this reflex is obtainable over a receptive field including the entire mesial

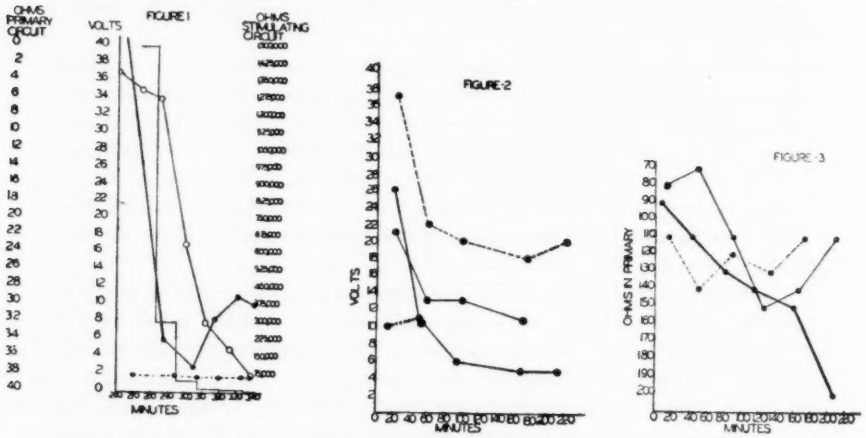


Fig. 1. Acute cat 6. Threshold of ipsilateral flexion reflex in a steady state four hours after transection. After each set of readings the electrode in the pad of the paw was inserted deeper; that in the thigh remained at a constant depth to control the reflex stability of the animal. Paw electrode indicated by solid lines; circles indicating single induction shocks; black points, repetitive stimuli from condenser; resistance of circuit through animal indicated by solid line in steps. Thigh electrode (giving ipsilateral extension) indicated by broken line. The figures for resistance are abnormally high because polarization was not avoided. They are valid only in relation to each other and not as absolute values. Note that the condenser threshold is lowest at an intermediate depth, presumably that of the receptors.

In all figures time is plotted as the abscissa from the moment of transection.

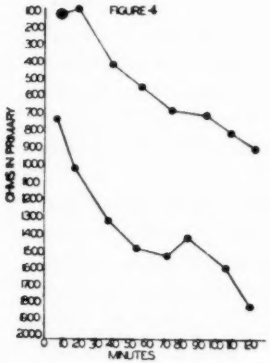
Fig. 2. Acute cat 5. Threshold of ipsilateral reflexes from alternating charge and discharge of 0.01 m.f. condenser, following transection under divinyl ether. Heavy, solid line (lowest) indicates hind paw electrode (flexion); light, solid line hind leg electrode (flexion); broken line, thigh electrode (extension); dotted line, forepaw electrode (flexion).

Fig. 3. Acute cat 7. Threshold of ipsilateral reflexes from repetitive induction shocks. Same symbols as in figure 2. Between the second and third stimulations of the paw, this electrode came out and was replaced. The resistance reading was identical before and after this adjustment and there is no break in the threshold curve. Hence the subsequent readings are considered comparable with the preceding ones.

aspect of the limb and extending up the lateral surface to a line about one-third of the way from the knee to the great trochanter. The thigh electrode was placed above this limit and induced ipsilateral extension consistently in all acute experiments. Ultimately the receptive field extends

farther up. In two cats it included the entire thigh for the first time seven days after transection. No gradation of threshold was detected that could be correlated with distance from the focal point of the receptive field. This statement must be accepted with reserve, since our method does not justify quantitative comparison of threshold at one electrode with that at another.

Local sign is well developed. In the acute cat the threshold response from mid thigh was noted only in extensors of the knee; that from mid leg only in flexors of the ankle; that from pad of paw only in plantar flexion of digits.



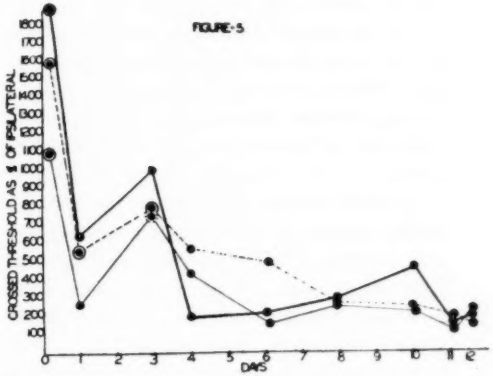


Fig. 4. Acute cat 9. Threshold from internal saphenous nerve for repetitive induction shocks. Lower line, ipsilateral flexion; upper line, crossed extension except for the first point which is crossed flexion. Note the slight rise between the first and second points. This is the earliest reversal of crossed response in any of our animals.

Fig. 5. Chronic cat 1. Crossed reflex threshold expressed as percentage of ipsilateral threshold. Electrodes on paw, leg and thigh indicated as in figures 2 and 3. Black dots surrounded by open circles indicate crossed flexion; dots without open circles, crossed extension; dot with broken circle, adduction and flexion followed by extension.

In pattern the ipsilateral flexion reflex is not invariably so stereotyped as the literature of the subject suggests. Under strong stimulation the following aberrant forms have been occasionally elicited: from the leg ipsilateral extension of thigh, flexion of knee, fanning of toes; from the toe pad ipsilateral flexion of knee and thigh, extension of ankle with crossed extension; and ipsilateral flexion of knee, extension of thigh and ankle with crossed flexion.

Turning to our chief interest, the question of a progressive change in threshold indicative of spinal shock, the early experiments in which transection was preceded by decerebration under diethyl ether proved irregular and inconclusive. Only when divinyl ether was substituted for diethyl

ether and decerebration was omitted, the curves of threshold plotted more smoothly. Of four such experiments, in which the same type of electrode was used as in the preceding series, all showed progressive fall in threshold at the paw and two of them also at the leg (figs. 2 and 3). Of two experiments in which Sherrington electrodes were placed on the internal saphenous nerve, one was vitiated by blood seeping into the electrode holder. The rise in threshold presumably reflects the short circuit at the electrodes rather than the state of the cord. This animal was decerebrated one minute before transection. The other cat with brain intact showed a typical picture of shock, the threshold falling steadily for both ipsilateral and crossed responses (fig. 4).

The reflex of ipsilateral extension from the thigh. This reaction, although occasionally showing a slight initial fall of threshold (fig. 2), is relatively free from shock in the cat (fig. 3). Usually it exhibits no significant change throughout the course of an acute experiment. After several days it is replaced by flexion through an extension of the receptive field of the flexion reflex.

Crossed reflexes. In no case have we found an interval after transection during which a crossed response could not be elicited. Initially the pattern is flexion. After an interval ranging from twenty minutes (acute cat 9, fig. 4) to more than twenty-four hours (chronic cat 2) this reflex is replaced by crossed extension. Both crossed flexion and crossed extension are frequently associated with adduction. During the period of transition the response may be a simple adduction or diphasic extension-flexion or flexion-extension. The receptive field for crossed extension emerges from complete shock first at the foot in response to strong stimulation. At this stage it is sometimes possible to elicit either crossed flexion or crossed extension at will, the former by a weak stimulus, the latter by a strong one. The receptive field extends rapidly up the leg. At the mid thigh, however, crossed flexion usually persists for a considerable time after both foot and leg are consistently yielding crossed extension. Thus in chronic cat 1, paw and leg gave crossed extension twenty-four hours after transection; thigh gave only crossed flexion until the fifth day. From that time onward the response from all three points was invariably crossed extension. Yet the receptive field for the crossed extension reflex invades the lateral aspect of the thigh much earlier than does that of the *ipsilateral* flexion reflex. Thus there is usually a considerable interval during which this region yields extension of both limbs. In chronic cat 1, crossed extension from the thigh appeared on the fifth day, ipsilateral flexion from the thigh on the seventh; and on the ninth and twelfth days this region again yielded bilateral extension. Similarly in chronic cat 2, on the fifth day the response from the thigh shifted from ipsilateral extension with crossed flexion to bilateral extension. Only on the seventh day did it elicit ipsilateral flexion for the first time.

Immediately after transection the threshold for crossed flexion may be from seven to eighteen times as high as that for ipsilateral flexion. Frequently it falls rapidly and may reach a value of about three times that of the ipsilateral reflex during the course of an acute experiment. The threshold for the crossed extension reflex continues falling for many days and may ultimately attain a level little higher than that for ipsilateral flexion (fig. 5).

Discussion. The validity of the threshold curves in the acute experiments hinges upon the rapidity of recovery from anesthesia. In every acute case in which divinyl ether was used, the cat was conscious within less than ten seconds after withdrawal of the anesthetic and after the longer aseptic operations within less than a minute. Goldschmidt, Ravdin, Lucké, Müller, Johnston, and Ruigh (5) state that in the human "the usual period for recovery to the stage of answering questions rationally after operations with a duration of from twenty to forty minutes was from thirty seconds to five minutes. In a patient with a breast carcinoma the duration of the operation was one hour and twenty-two minutes, and the time from complete withdrawal of the anesthetic to recovery was twenty seconds." In the monkey their longest anesthesia was six hours. This animal was on its feet and eating a banana within six minutes after withdrawal. In our acute experiments anesthesia usually lasted less than twenty minutes. We feel confident, therefore, that it had no influence upon determinations of threshold.

Decerebration, on the other hand, we regard as the source of variations in threshold of considerable magnitude. Even when the interval between decerebration and transection is too brief for much recovery from shock in the interoperative period, there may be enough shift from fall of temperature or other undetected factors to mask recovery from a mild degree of reflex depression. Of five such experiments only two yielded unequivocal evidence of shock; of five acute animals which were not decerebrated, all showed a progressive fall in threshold.

To us at least, the most surprising feature of our results is the very slight degree to which the reflex threshold of the cat is affected by spinal shock. Were the attempt made to relate the intensity of shock quantitatively to the degree of suprasegmental development in frog, cat, monkey, ape, and man, the member of the series that would depart farthest from a linear relation would be the cat. Whether this impression is exaggerated by the use of threshold rather than maximal responses we are not prepared to say. There is a feature of spinal shock first observed by Sherrington (8) which suggests such a possibility. In the monkey, when the ipsilateral flexion reflex is beginning to return, a weak stimulus may prove as effectual as a very strong one. The units of low threshold are less depressed than those of high threshold. Whether the same phenomenon obtains to a

lesser degree in the cat we are as yet unable to say. If so, it is so slight that the myograph would be required for its demonstration. Unfortunately, the preparation for myographic recording is too long to permit its use during the initial period.

Perhaps our most important observations concern the sequence of recovery of the crossed responses. Although we had not infrequently encountered crossed flexion in the acute spinal cat prior to the present research, we had not previously realized that it is virtually invariably present immediately after transection. We suspect the stigmatic electrode may afford a peculiarly adequate stimulus for this reflex. We have observed at a time when the crossed response to stigmatic stimulation was still uniformly flexion, that gentle squeezing of the entire foot induced crossed extension. Our earliest instance of crossed extension in the cat was from stimulation of a nerve trunk (cat 9, fig. 4). It should be emphasized that these remarks refer solely to cat. In the dog, also, we have obtained crossed responses immediately after transection under divinyl ether; but these have invariably been crossed extensions. Occasionally, in the dog there is an interval of several minutes after transection during which no crossed response can be obtained, but this absolute phase of shock to the crossed reflex was found in only one out of three cases. In the cat, on the other hand, although crossed flexion is present immediately after transection, we have not seen crossed extension in under twenty minutes and sometimes not for more than twenty-four hours. It would seem an obvious inference that the longer interval in cat than dog before crossed extension is elicitable is due to its inhibition in the former animal by a reflex of crossed flexion, which is not present in the latter. This view accords with the interpretation advanced by Matthes and Ruch (6) of the recruitment of the crossed extension reflex in the chronic spinal cat.

CONCLUSIONS

Divinyl ether, due to its rapid elimination, is a suitable anesthetic for transection designed for the study of the initial phase of spinal shock.

The transient depression following spinal transection under divinyl ether in the full grown cat involves the reflex thresholds studied in the following order of severity; ipsilateral extension elicited from the external aspect of the thigh, little or not at all; ipsilateral flexion, relatively little; crossed flexion, moderately; crossed extension, severely.

Of these reflexes, the first three are present immediately after transection. Crossed extension may appear at any interval from twenty minutes to more than twenty-four hours. The lateness of its appearance is probably due partly to spinal shock, partly to inhibition from the crossed flexion reflex. Similarly, the ipsilateral flexion reflex, though relatively little affected by shock, does not extend its receptive field effectively over

that of the still less involved antagonistic reflex from the external aspect of the thigh for several days after transection.

REFERENCES

(1) Forbes, A. Physiol. Reviews **2**: 361, 1922.
(2) Forbes, A. and P. C. Baird, Jr. This Journal **87**: 527, 1929.
(3) Forbes, A., S. Cobb and H. Cattell. This Journal **65**: 30, 1923.
(4) Fulton, J. F. and C. S. Sherrington. J. Physiol. **75**: 17, 1932.
(5) Goldschmidt, S., I. S. Ravdin, B. Lucké, G. P. Müller, C. G. Johnston and W. L. Ruigh. J. A. M. A. **102**: 21, 1934.
(6) Matthes, K. and T. C. Ruch. Quart. J. Exp. Physiol. **22**: 221, 1932.
(7) McCouch, G. P. This Journal **71**: 137, 1924.
(8) Sherrington, C. S. Phil. Trans. Roy. Soc. B **190**: 45, 1898.
(9) Sherrington, C. S. J. Physiol. **40**: 66, 1910.
(10) Sherrington, C. S. and S. C. M. Sowton. J. Physiol. **49**: 331, 1915.

THE SYNAPTIC DELAY OF THE MOTONEURONES[1]

R. LORENTE DE NÓ

From the Central Institute for the Deaf and the Department of Otolaryngology, Washington University Medical School, St. Louis, Missouri

Received for publication November 26, 1934

It is generally accepted that the response of the motoneurones to excitatory impulses does not appear immediately; a certain time, the synaptic delay, elapses between the arrival of the excitatory impulses at and the firing off of the motoneurone. This assumption is based upon the fact that the reduced latent period of any reflex, i.e., the observed latent period less conduction time in the afferent and efferent paths always is found to be a measurable interval of time.

However, the duration of the synaptic delay has not been accurately determined yet. On one hand the calculation of the conduction time is made difficult by the fact that the speed of conduction in the nerve fibres of the same nerve varies between very wide limits (Erlanger, Gasser and Bishop, 1926; Erlanger and Gasser, 1929; Bishop and Heinbecker, 1930; Bishop, Heinbecker and O'Leary, 1932; Blair and Erlanger, 1933) and in general it is not known which particular type of fibres is involved in the production of the reflex. This cause of error has been largely eliminated in the experiments of Eccles and Sherrington (1931a), who actually measured the speed of conduction of the afferent and efferent waves and in the experiments of Gasser and Graham (1932), in which the potentials developed in the spinal cord were recorded. These authors found potentials associated with reflexes 2.2 to 3.6 σ after arrival of the excitatory impulse. These figures are somewhat lower but not very different from the reduced latent period of the flexor reflex (3 to 5σ) as calculated by Jolly (1911), Forbes and Gregg (1916) and Eccles and Sherrington (1931a). However, this figure is not the shortest possible central delay because the response to a second shock arriving at a favorable interval after the first may have a delay of only 0.2 to 0.4 σ (Eccles and Sherrington, 1931b).[2]

[1] The work reported in this paper has been aided by a grant of the Rockefeller Foundation.

[2] After completion of the present work, C. E. Leese and L. Einarson published a very interesting paper (This Journal **109**: 296, 1934), in which the problem of the latent period in the spinal flexor reflex has been examined again. They find discrepancies between their observations and those of the Oxford school which may be attributable to variability in the state of the centres and to difficulties in calculating conduction time. They obtain a lower figure (2σ) for the reduced latent period of the flexor reflex.

The great variability of the central reflex time is in contrast with the constancy of the time relations in nerve and muscle activity so that one is inclined to think that it is due rather to the properties of groups of neurones than to the properties of the individual neurones. It seemed therefore advisable to devise preparations which would eliminate the internuncial neurones between the stimulated fibres and the motoneurones and in which the paths for the afferent and efferent impulses are such that conduction times do not need to be considered.

The terminal part of the reflex arcs ending on the motoneurones of the eye muscles offer the possibility of obtaining such a preparation.

In the rabbit there are three main reflex arcs ending on the motoneurones of the eye muscles: the vestibular arc, which sets up the labyrinthine reflexes, the trigeminal arc, which sets up the (protective) retraction reflex of the eye (a part of the corneal or lid reflex) and the deep neck reflex arc which sets up the tonic (Bárány 1907) and dynamic (Lorente de Nó, 1927, 1931) cervical reflexes on the eyes. All these arcs are similarly built, they contain paths going without interruption to the motor nuclei and other paths including one or more internuncial neurones (Lorente de Nó, 1933a, b).

It is a fortunate circumstance that in the proximity of the abducens nucleus practically all the paths going to the oculomotor nuclei are collected in a small region close to the floor of the fourth ventricle, so that if electrodes are placed on or near to the abducens nucleus the electric shocks will excite simultaneously the fibres of the abducens nerve and fibres going to the third nucleus, thus stimulating its motoneurones across one synapse. Since the length of the fibre paths from the point of stimulation to the external and the internal rectus muscles are very nearly the same and the speed of conduction at least of the fastest fibres also has to be very nearly the same (see later) the delay in response of the internal rectus muscle will measure the synaptic delay. Figure 1 shows diagrammatically the anatomic relations.

TECHNIQUE. All the experiments have been carried out with rabbits. Under deep anesthesia the cerebral cortex and the cerebellar vermis were extirpated thus making the floor of the fourth ventricle accessible. Care was taken to cut the cerebellar vermis medial to the vestibulo-cerebellar complex thus avoiding lesions of the primary vestibular nuclei. The carotids were not ligated. The eye muscles were prepared and all but the horizontal ones and all the tissues in the orbit were removed in so far as this was compatible with a good blood supply of the remaining two muscles. The anesthesia was discontinued.

The horizontal muscles (*Rectus externus* innervated by the abducens nerve and *Rectus internus* innervated by the oculomotor nerve) were connected to isometric myographs for recording their contractions on photo-

graphic paper. (Shortening of the muscle 0.2 mm. per gram tension, initial tension 2 to 3 grams, tension developed by maximal twitch of recently prepared muscle 2.5 grams, speed of the camera up to 30 cm. sec.)

Three electrodes were placed on the muscles. The grid electrode close to the ligature on the tendon, and the recording ground one 8 mm. more proximal; especial care was taken in placing the ground electrodes in both muscles at the same distance from the end of the muscle and therefore from the entrance of the nerve. Repeated control experiments showed that in most cases the action potentials of twitches set up by shocks delivered to the motor nerves were perfectly synchronous in both muscles, in a few cases the difference was a few hundredths of 1σ and only in one case was the difference as large as 0.1σ. The third electrode was a resistanceless ground as proposed by Bishop in order to reduce the electric artefact.

The recording electrodes fed into an amplifier which drives simultaneously a galvanometer writing on photographic paper and a cathode ray oscillograph. The recording and stimulating apparatus were described in another paper (Blossom and Lorente de Nó, 1934), so that it is only necessary to mention here that the stimulating shock was produced by the discharge of a condenser, generally about $0.1\mu f$ through an induction coil (Schmitt and Schmitt, 1932). The duration of the shock as measured with the cathode ray oscillograph was 0.07σ. Across the secondary coil there was a 1000-ohm potentiometer to regulate the strength of the shock. Across the potentiometer appeared 20 volts; threshold excitation of the external rectus muscle from the floor of the fourth ventricle was generally obtained at 0.06 volt, often at 0.04 and never at more than 2 volts.

The bipolar stimulating electrodes were of platinum wire (distance between points 0.5 to 1 mm.). The wires were insulated in their entire length but the very point.

In the first experiments great difficulty was found in keeping the muscle at normal temperature and especially in preventing it from drying, so that the observations were only reliable when made during the first hour after the operation. Later a simple procedure was devised which allowed the maintenance of the muscle in good condition for several hours. A vaporizer was placed at such a distance from the muscle that this was surrounded by water vapor at 37°C. Under such conditions and if the muscle was under an initial tension no larger than 2 grams, the tension developed by the twitch, the action potential and the latent period of the response remained constant within very narrow limits for many hours.

Results. When the stimulating electrodes are placed on or close to the abducens nucleus (position A, fig. 1) the external rectus muscle on the same side answers to single shocks with twitches exactly like those produced when the electrodes are placed on the abducens nerve. The tension and

potential developed are proportional to the strength of the stimulus. The latent period is the sum of conduction time in the motor fibres and endplate delay. The height of the twitch is independent of the state of the centres so that it remains constant during nystagmus (fig. 2, II) and only when added to pre-existing strong contractions does it diminish slightly. This effect is comparable to the "busy line" effect described by Forbes, Whitaker and Fulton (1927). All this shows that the electric shock has excited the fibres of the abducens nerve or that if the excitation has been of the motoneurones, these when thus excited set up the impulse in the axon without appreciable latency.

The internal rectus muscle also answers with a twitch (fig. 2), which however differs from that of the rectus externus in three fundamental features; *a*, it appears after a long and variable latency; *b*, it may be asynchronous, the synchronization being dependent on the state of the centres; *c*, its height depends very much on the state of the centres.

There are preparations in which weak stimuli set up very strong contractions of the internal rectus muscle, even maximal twitches and preparations in which strong stimuli set up small contractions. In three out of about thirty preparations it was impossible to elicit any response to single shocks of the internal rectus muscle although the external rectus one readily responded to weak shocks (0.5 volt). This effect is comparable to the inhibition of the internal rectus muscle during vestibular reflexes (Lorente de Nó, 1928, 1931, 1933a) due to the functional status of the reticular substance.

But the dependence of the response of the internal rectus muscle on the state of the centres is best demonstrated when the stimulation is produced during nystagmus. As figure 2 shows the twitch is large during the contraction phase and small during the relaxation one. Occasionally no response at all appears when the muscle is totally relaxed.

This is best explained by assuming that during nystagmus as well as during spinal reflexes a "subliminal fringe" (Denny Brown and Sherrington, 1928; Sherrington, 1929) is present, composed of subliminally excited motoneurones, which therefore have a lower threshold. The greater the contraction the greater will be the subliminal fringe and therefore the facilitated twitch.

The latency of the response of the external rectus muscle is the sum (fig. 1) of the conduction time in the sixth nerve, the endplate delay and the conduction time in the muscle. The latency of the internal rectus muscle when excited through path *a* (and it will be shown later that the responses dealt with in this paper are so produced) is the sum of the conduction time in path *a*, the synaptic delay in the motoneurones, the conduction time in the third nerve, the endplate delay and the conduction time in the muscle. Since the length of both fiber paths is very nearly the

same and the endplate delays and conduction velocity in both muscles are alike it is evident that the difference between the latencies of both responses will be due only to the synaptic delay in the motoneurones of the third nucleus.

The data obtained in thirteen well-controlled experiments (see figs. 1 and 2 in the following paper) have been collected in table 1. If the experiment of 6/19/34 is excluded it can be said the values of the synaptic delay

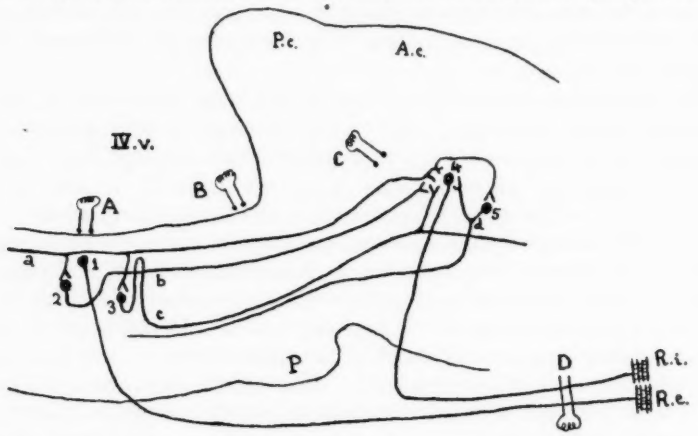


Fig. 1. Diagram showing the anatomic relations in the preparation used in the experiments. The basis is a median longitudinal section through the brain of the rabbit, without cerebellum. *M.*, medulla; *P.*, pons; *P.c.*, posterior colliculus; *A.c.*, anterior colliculus; *IV. V.*, fourth ventricle; *1*, motoneurones of the abducens nerve innervating the external rectus muscle (R.e.); *2*, and *3*, neurones of the reticular substance; *4*, motoneurones of the oculomotor nerve innervating the internal rectus muscle (R.i.); *5*, neurones of the reticular system in the midbrain; *a*, a fibre representing the long pathways (posterior longitudinal bundle, tractus predorsalis, etc.) from the vestibular, trigeminal and cervical nuclei; fibres *b* and *c* represent the reticular paths which cross the middle line and are placed near to the floor of the ventricle; fibre *d* represents descending reticular paths which, although few in number, give off collaterals to the oculomotor nuclei. *A*, *B*, *C*, *D*. Positions of the stimulating electrodes.

are surprisingly constant. The minimal duration is 0.5σ and the maximal duration 1.36σ. The maximal variation of the endplate delay is of the same order of magnitude (0.7 to 1.3σ, expt. of 6/3/34).

The variation of the synaptic delay is accompanied by a variation of the developed potential, i.e., of the number of active motor units. Curves I and II (fig. 3) were obtained with resting preparations, i.e., preparations without spontaneous nystagmus. These curves are similar to the curves published by Eccles and Sherrington (1931a) relating the developed tension

to latent period in the case of the flexor spinal reflex and the curves published by Lorente de Nó (1933c) relating tensions and latent period in the acoustic tensor tympani reflex. However, no great significance can be attached to the similarity because these curves can not yet be analyzed mathematically.

For instance, the curves in figure 3 embody besides the function relating

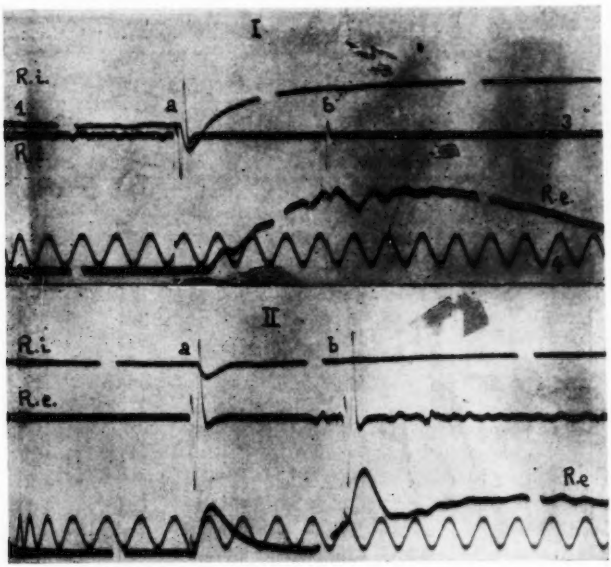


Fig. 2. Responses of the horizontal eye muscles to single shocks. Electrodes on the floor of the fourth ventricle. Submaximal shocks. *R. e.*, rectus externus; *R, i.*, rectus internus; lines *1* and *2* are isometric myograms. Line *3* is a galvanometer record of the action potential of one of the muscles. Line *4* is a record of the time ($\frac{1}{10}$ sec.) and of the stimulus. The muscles were having nystagmus due to an accidental lesion of the vestibular nuclei. In *I*, immediately after the operation, the electrodes were on the pons close to the middle line, but some 4 mm. oral to the abducens nucleus (position *B*, fig. 1). The external rectus muscle did not respond. The response of the internal rectus muscle was in *a*, a very large twitch, the muscle was at that moment in the maximum of the contraction phase of the nystagmus. In *b*, the response of the muscle was scarcely visible (the deflection of the galvanometer was due to shock artefact); the muscle was then at the maximum of the relaxation phase of the nystagmus.

In *II*, half an hour later, the electrodes were placed on the abducens nucleus. The external rectus muscle responded with maximal motor twitches which were not affected by the nystagmus. The response of the internal rectus muscle was in *a*, during the contraction phase, large, and in *b*, during the relaxation phase, very small. The nystagmus was beginning to subside. Experiment of 5/13/34.

duration of the synaptic delay to strength of stimulus (number of active synapses) two other statistical functions; one of them is of distribution of thresholds of the stimulated fibres and will remain constant within narrow limits (Blair and Erlanger, 1933), and the other is of distribution of thresholds of the stimulated neurones. The existence of this second function and its dependence on the state of the centres is shown in figure 2.

The curves B in figure 3 (I and II) demonstrate that near threshold the twitch is asynchronous, if the strength of the stimulus increases the discharge is synchronized, at the same time that new slow reacting motor-

TABLE 1

Latencies of the responses of the horizontal eye muscles

Time in σ

DATE	ELECTRODES ON THE FLOOR OF THE FOURTH VENTRICLE		ELECTRODES ON THE MOTOR NERVES IN THE CRANIAL FOSSA		SYNAPTIC DELAY IN THE MOTONEURONES
	R. internus	R. externus	R. internus	R. externus	
			0.9 (1)		
6/ 3/34	1.85		1.5 (2)	0.9	0.85
6/ 4/34	1.9 to 2.25				
6/ 5/34	1.70 to 2.10		0.9 to 1.00	0.95 to 1.10 (3)	0.60 to 1.00
6/ 8/34	1.75 to 1.87				
6/12/34	1.75				
6/15/34	1.60 to 2.25				
6/16/34	1.60 to 2.20	1.10	0.90		0.50 to 1.10
6/18/34	1.80 to 2.25	1.05	1.00	1.00	0.75 to 1.25
6/19/34	2.50 to 3.00	1.50(4)	0.90	0.85	1.5 to 2.00
6/20/34	1.65 to 1.80	1.10			0.55 to 0.70
6/21/34	1.75 to 2.00	1.06			0.69 to 1.14
6/24/34	1.70 to 2.20	0.86			0.84 to 1.36
6/25/34	1.60				

Observations. 1, rabbit alive, muscle at normal temperature; 2, rabbit dead, muscle cool; 3, variations due to stimulus escape; 4, the electrodes were on the pons and therefore the muscle was being excited across its motoneurones, synaptic delay 0.65σ; with the electrodes introduced in the colliculus posterior the latency of the response of the internal rectus muscle dropped to the ordinary figure of 1.60 to 1.75σ.

neurones are brought into activity; with strong stimuli the twitch often becomes synchronous and can not be differentiated from a motor twitch. That the synaptic delay decreases when the strength of the stimulus increases seems evident although the exact relation between both can not be ascertained yet.

Controls. a. That the reaction of the internal rectus is due to excitation of the motoneurones and not to spread of the stimulus to the oculomotor nerve is shown by the following facts: 1, it may vary in latency and strength independently of the stimulus; 2, if a transversal lesion of the

pons is made cutting the paths between the stimulated point and the oculomotor nucleus, the reaction disappears, to reappear again when the electrodes are placed between the lesion and the motor nucleus of the third nerve; 3, when the electrodes are placed on the motor nerves (III and VI) in the cranial fossa (position D, fig. 1) both muscles respond at the same time, while with the electrodes placed on the floor of the fourth ventricle (position A, fig. 1) the internal rectus muscle shows a considerable delay; 4, the response of the external rectus muscle can be elicited after death of the rabbit, whereas that of the internal rectus muscle disappears while the

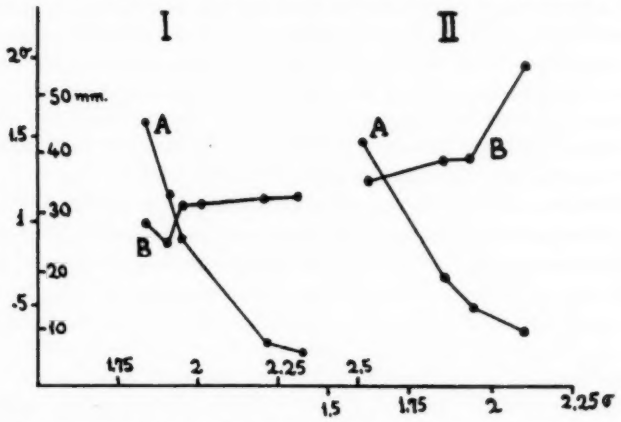


Fig. 3. Stimulation of the internal rectus muscle across its motoneurones. Shocks of variable intensity. The abscissae show the latent period of the response in sigma. Given in ordinates: curves *A* potential of the twitch, scale in millimeters at left; sensitivity 12 mm. per millivolt; curves *B* time to crest of the action potential, descent of the curve indicates synchronization of the response. Curves I experiment of 6/18/34. Curves II experiment of 6/15/34. Stimulating electrodes on the floor of the fourth ventricle, on the abducens nucleus of the same side (position A, fig. 1). Potentiometer settings: Curves 1; 0.6; 0.4; 0.3; 0.2; 0.1. Curves II: 0.2; 0.1; 0.07; 0.05; potential across the potentiometer about 20 volts.

heart is still beating; therefore the response of the internal rectus muscle shows a great dependence on the blood supply of the centres; 5, it is possible, too, to excite the external rectus muscle across its motoneurones. When the electrodes are placed some 4 mm. oral to the abducens nucleus (position B, fig. 1) the external rectus muscle either does not respond at all as in figure 2, *1* or responds after a delay (0.65σ) comparable to the synaptic delay in the motoneurones of the oculomotor nucleus. If the electrodes are brought back on the abducens (position A, fig. 1) the motor twitches reappear again (fig. 2, *2*).

b. Another possible cause of error is that the conduction velocity in

fibres *a* (fig. 1) may be different from that of the motor fibres. The shortness of the path (about 10 mm.) would make the error at any rate insignificant but besides it is possible to show that at least the fibres with low threshold in paths *a* must conduct with a speed very similar to that of the fast motor fibres.

An actual measurement of the speed of conduction has not yet been possible. But the measurement of the refractory period is not difficult (Lorente de Nó, 1935 b). The consistent results of several experiments allow of fixing about 0.6σ as the lowest limit of the end of the absolutely refractory period, which brings the fastest among the stimulated fibres in the class α of the A fibres. The fast motor fibres of the abducens nerve also conduct with a speed of 80 to 90 M.P.S. This has been determined with some accuracy by measuring the difference in latency of the muscle response when the electrodes were placed at three different points of the abducens nerve (on the floor of the fourth ventricle, at the level of the emergence of the oculomotor nerve and at the entrance in the orbita).

Discussion. It is to be considered now whether neurones *2*, *3* and *5* (fig. 1) have had any participation in the production of the twitch of the internal rectus muscle. That these neurones also are excited and brought into activity will be shown in another paper (Lorente de Nó, 1935b) but this activity does not shorten the synaptic delay of the motoneurones as measured in responses to strong shocks. With maximal facilitation the synaptic delay is never reduced below the minimal figure in table 1 and with strong inhibition is never prolonged by more than 60 per cent of the minimal value in the same preparation. Since the fibres *a* are always stimulated and a strong facilitating discharge of neurones 2, 3 and 5, such as is present during nystagmic contractions, does not reduce the latent period more than an increase in the strength of the stimulus, it is evident that the reaction to single shocks is primarily produced by the *a* fibres (fig. 1). But a conclusive proof is found in the fact that the synaptic delay is very nearly the same when the electrodes are placed on the pons close to the entrance to the Aqueductus Silvyi (position *B*, fig. 1). In this case the stimulus excites fibres *a* and the axon of the greatest part of cells 2 and 3 which as shown in other papers (Lorente de Nó, 1922, 1933a, b) are collected in the proximity of the abducens nucleus.

In only one experiment (6/19/34) an abnormally high latent period was found 2.5 to 3σ (synaptic delay 1.5 to 2σ) but in this case there were good reasons to believe that the *a* fibres alone were not able to produce a response without activity of the cells *2* and *3*. In this experiment a crucial test was made. The electrodes were placed first very close to the entrance of the Aqueductus Silvyi (position B, fig. 1); this produced a noticeable shortening of the delay (unfortunately not measured) and then were introduced to a depth of about 4 mm. in the posterior colliculus towards the oculo-

motor nucleus (position C in fig. 1) so that most of the fibres entering the oculomotor nucleus had to be excited by the shock. The latency (repeatedly measured) dropped to the ordinary figure of 1.6 to 1.75σ (synaptic delay 0.7 to 0.85σ).

There can be no doubt that the twitch observed in all the other experiments has been a response to the stimulation of the *a* fibres (fig. 1) and that the synaptic delay of the motoneurones even when facilitated is never less than 0.5σ, a figure remarkably close to the endplate delay as measured in the eye muscles.

However this does not mean that other neurones may not have a longer or shorter synaptic delay. Other experiments (Lorente de Nó, 1935b) seem to show that the internuncial neurones in the medulla have a similar synaptic delay (about 0.6σ) but these neurones also belong to the same histological type as the motor ones. For the sympathetic neurones the values found by Bishop and Heinbecker (1932) are decidedly higher but recently Brown (1933) and Eccles (1933) have found much earlier responses.

To determine the origin of the synaptic delay is a difficult problem. It seems to be of the same order of magnitude as the muscle endplate delay and can therefore be explained by the assumption that in exciting a new cell the impulse, on account of its wavelike nature, needs a long utilization period.

CONCLUSION

The synaptic delay in the motoneurones of the third nerve has been measured by means of preparation in which no internuncial neurones are present between the excited fibres and the motoneurones and in which errors due to unknown speeds of conduction can not occur.

The synaptic delay measures from 0.50 to 1.36σ and it is roughly in inverse relation to the strength of the stimulus (number of activated synapses).

The endplate delay in the eye muscles measures from 0.7 (in normal) to 1.3σ (in cool muscle).

REFERENCES

BÁRÁNY, R. Zentralbl. Physiol. **20**: 298, 1907.
BISHOP, G. AND P. HEINBECKER. This Journal **94**: 170, 1930.
This Journal **100**: 519, 1932.
BLAIR, E. A. AND J. ERLANGER. This Journal **106**: 524, 1933.
BLOSSOM, H. AND R. LORENTE DE NÓ. Acta Otolaryng. In press.
BROWN, D. AND C. S. SHERRINGTON. J. Physiol. **66**: 175, 1928.
BROWN, G. L. J. Physiol. **81**: 228, 1934.
CAJAL, S. R. Beiträge zur Kenntniss der Medulla oblongata, etc. Leipzig. 1896.
Histologie du systeme nerveux. I. Paris. 1909.
ECCLES, J. C. J. Physiol. **80**: 248, 1933.

ECCLES, J. C. AND C. S. SHERRINGTON. Proc. Roy. Soc. *B*.**107**: 511, 1931a.
Proc. Roy. Soc. *B*.**107**: 535, 1931b.
ERLANGER, J. AND H. S. GASSER. This Journal **92**: 43, 1930.
ERLANGER, J., H. S. GASSER AND G. BISHOP. This Journal **78**: 537, 1926.
FORBES, A. AND A. GREGG. This Journal **37**: 118, 1916.
FORBES, A., L. R. WHITAKER AND J. F. FULTON. This Journal **82**: 693, 1927.
GASSER, H. S. AND H. T. GRAHAM. This Journal **103**: 303, 1932.
HEINBECKER, P., J. O'LEARY AND G. H. BISHOP. This Journal **104**: 23, 1933.
JOLLY, W. A. Quart. J. Exp. Physiol. **4**: 67, 1911.
LORENTE DE NÓ, R. Acta Otolaryng. **11**: 301, 1927.
Die Labyrinthreflexe auf die Augenmuskeln, etc. Wien, 1928.
Ergebn. Physiol. **32**: 73, 1931.
Arch. Neurol. and Psych. **30**: 245, 1933a.
The Laryngoscope **43**: 1, 1933b.
Trans. Am. Oto-rhino-laryngol. Soc. Chicago, 1933c.
The refactory period of the motoneurones. To be published. 1935a.
Facilitation and inhibition of motoneurones. To be published. 1935b.
SCHMITT AND SCHMITT. Science **76**: 328, 1932.
SHERRINGTON, C. S. Proc. Roy. Soc. *B*.**105**: 332, 1929.

THE REFRACTORY PERIOD OF THE MOTONEURONES[1]

R. LORENTE DE NÓ

From the Central Institute for the Deaf and the Department of Otolaryngology, Washington University School of Medicine, St. Louis, Missouri

Received for publication November 26, 1934

The determination of the refractory period of the motoneurones by means of two successive stimuli applied to the afferent path of a reflex arc offers very serious difficulties, because the first shock facilitates or inhibits a certain number of neurones, thus modifying the responses to the second one. Eccles (1931) considering the difficulties of the problem, used antidromic shocks to exclude the possibility of the second response being due to motoneurones which were not made refractory by the first shock. He decided that the absolutely refractory period can not be longer than 2.4σ and the relatively refractory one longer than 14σ.

The preparation described in the preceding paper (Lorente de Nó, 1935a) made it possible to demonstrate a much lower limit for the refractory period of the motoneurones.

TECHNIQUE. The preparation has been the same as in the preceding paper. The shocks have been delivered by two Thyratron tubes discharging through the same coil. The first (conditioning) shock is produced by the discharge of a condensor of $0.13\mu f$ or more. The second (testing) shock by the discharge of a $0.1\mu f$ condensor. Both shocks are alike when the first condensor has a capacity of $0.11\mu f$ so that in every experiment the first shock has been decidedly larger. This has been tested both electrically (measurement of the voltage by means of the cathode ray oscillograph) and biologically (by means of the nerve muscle preparation).

RESULTS. The delay of the response to a second shock in the nerve muscle preparation has been studied by Lucas (1911) and by Forbes, Ray and Griffith (1923) who extend and support Lucas' findings and interpret Samojloff's results as due to some technical cause of error, an opinion also expressed by Gasser and Erlanger (1925). In the case of nerve the same problem has been investigated by Gotch (1909), Forbes, Ray and Griffith, and Gasser and Erlanger (1925).

The results obtained by these authors have been fully confirmed in the nerve muscle preparation. Figure 1 illustrates typical records. If

[1] The work reported in this paper has been aided by a grant from the Rockefeller Foundation.

plotted according to Lucas' method a curve of his type *c* is obtained, which shows that the second response is delayed during the relatively refractory period of nerve and muscle.

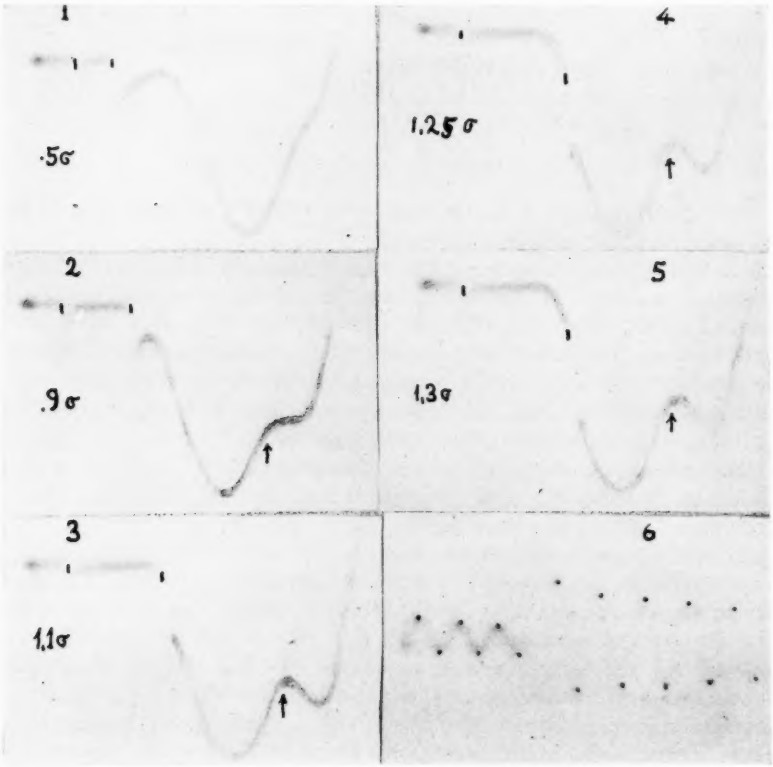


Fig. 1. Experiment of 6/21/34. Stimulating electrodes on the floor of the fourth ventricle close to the abducens nucleus. Stimulation of the abducens nerve by two submaximal shocks, the second smaller than the first one. The records are contact prints, on paper, of the action potentials of the responses of the external rectus muscle, as they appeared on the face of the cathode ray oscillograph at a sensitivity of 12 mm. per millivolt. Negative wave downwards. The distance between shocks is indicated on each record. (Records retouched for publication.) *1*. Shocks at 0.5 σ interval, the second shock is ineffective. *2*, *3*, *4* and *5*, shocks at 0.9; 1.1; 1.25; and 1.3 σ intervals, the second shocks sets up a delayed response which begins to appear in the descending (ascending in the prints) phase of the negative wave. The second response increases in size and has a shorter latency while it travels through the descending phase of the negative wave. *6*. Damped oscillatory discharge (2000 cycles per second) repeated 120 times per second. The sweep circuit is linear but owing to the properties of the tube seems to be slightly accelerating.

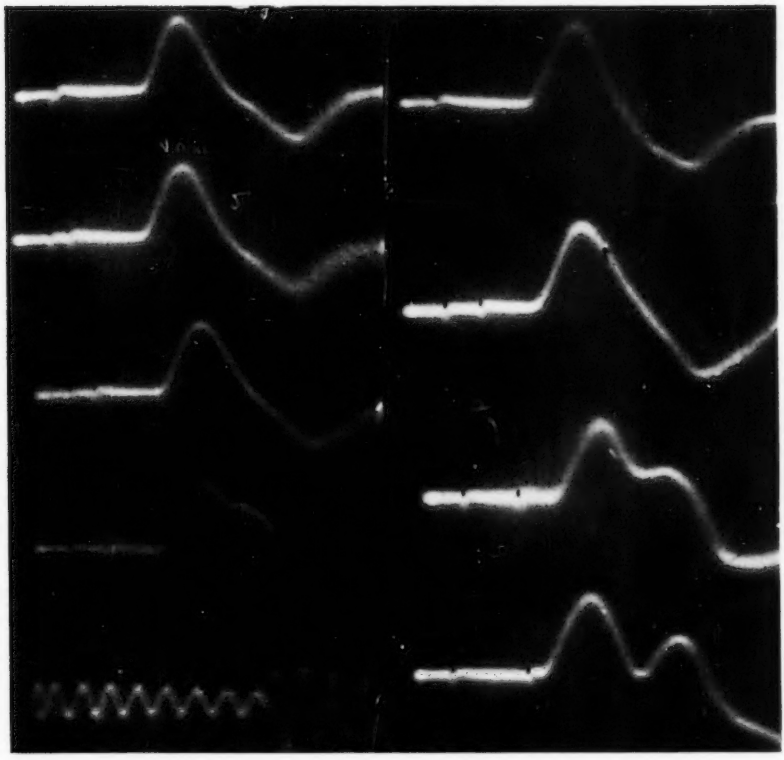


Fig. 2. Experiment of 6/25/34. Stimulating electrodes on the floor of the fourth ventricle close to the left abducens nucleus. Stimulation of the left internal rectus muscle across its motoneurones by two maximal shocks, the second one being smaller than the first. Contact prints on film (negativity upwards); sensitivity 1.5 mm. per millivolt. Series II of records was obtained 1½ hour after the first one and the recording electrodes on the muscle were rearranged; this accounts for the larger size of the potential wave and shock artefact in series II. The time scale is the same for both series. The distance between shocks is marked on each record.

Records *1* in both series contain only the first shock and show the form of the potential of the twitch as recorded in the particular position of the recording electrodes in each case. The comparison of these records with the lower ones shows the exact beginning of the second response. In records *2* and *3* of series I the second shock is ineffective. In record *4* there appears a second response after a considerable latency. In records *2*, *3* and *4* of series II there is a second response, the latency of which diminishes from record *2* to record *4*, but even in this last one the second response is considerably delayed.

The difference in latency of the response to the first shock in figures 1 and 2 measures the synaptic delay in the motoneurones of the third nucleus.

When the internal rectus muscle is being excited across its motoneurones repetitive responses of the muscle fibres will appear in every experiment because the neurones responding to the first shock have of course the lowest threshold and will therefore respond again as soon as they recover. However, the demonstration of the existence of a repetitive response can be made only in favorable cases, i.e., when the whole muscle or the greatest part of it has responded to the first shock, so that the second shock does not find any non-refractory motoneurone or else only those of very high threshold, which in spite of any possible facilitation are excited subliminally and do not respond.

In four well controlled experiments pure repetitive responses have been demonstrated. The observations were made as follows: With a relatively weak second shock the strength of the first one (regulated by the first condensor in the stimulator) and the interval between shocks setting up maximal facilitation of the second response were determined. The strength of both shocks was then increased until the second response was unaffected by the first shock. At that moment the second response was a maximal twitch which of course could not be increased by facilitation. The potentiometer setting was then further increased and the intensity of the first shock was made considerably larger than that of the second one. Under such conditions the first shock was setting up a maximal muscle twitch and any response to the second shock had to be repetitive. Records of the responses at various intervals between shocks were taken and immediately afterwards stimulating electrodes were placed on the oculomotor nerve. Maximal motor shocks were found to set up a muscle twitch developing the same potential as when the nerve was being stimulated across its motoneurones; but the latency of the response was of course shorter by an amount equal to the synaptic delay in the motoneurones.

There are considerable differences between facilitated and repetitive responses. Facilitated neurons give a response which may appear at any place of the action potential of the first response (fig. 1 in Lorente de Nó, 1935 b) even in the rising phase of the negative wave, while repetitive responses begin to appear at the same point of the descending phase of the negative wave where the first repetitive response is found in the nerve muscle preparation (figs. 1 and 2). Another important difference consists in that the facilitated response appears after a latency never longer than the latency of an equally strong response to the first shock. Even when the facilitated response is very small its latency is not appreciably lengthened. But the latency of the repetitive response is always longer than the latency of the first one by an amount equivalent to the delay of the second response in the partially refractory nerve-muscle preparation.

Figure 2 contains several records obtained in one of the experiments. They are extremely similar to those in figure 1; however, there is an im-

portant difference. In the ordinary nerve muscle preparation the second shock begins to be effective when placed at a distance of 0.85σ to 0.90σ after the first one. But when the excitation is being produced across the motoneurones the earliest effective second shock is found at approximately only 0.6σ after the first one. The comparison of records *I,3* and *II*, 2 of figure 2 with record 2 in figure 1 demonstrates that the second impulse must have appeared in the motor nerve no later than 0.85 to 0.90σ after the first one, because if it were not so the second muscle response would have been found later in the descending phase of the negative wave. Stimuli at shorter distance will have been ineffective on the muscle. Therefore, it has to be concluded that while passing through the synapses, dendrites and neurone body, the second impulse has been delayed about 0.25 to 0.30σ.

The fiber path between the stimulated point and motoneurones is so short (less than 10 mm.) that the second impulse must have reached the synapses and motoneurones without appreciable delay (Gasser and Erlanger, 1925); since the second impulse actually excited the motoneurone it has to be concluded that the absolutely refractory period was over. This enables me to fix 0.6σ as the upper limit of the absolutely refractory period of the motoneurones, i.e., the *absolutely* refractory period of the axon itself.

Discussion. There are some differences of opinion about the cause of delay of the response during the relatively refractory period of nerve. Gotch and Gasser and Erlanger believe it to be due only to slowed conduction; but Forbes, Ray and Griffith accept a dual process. On one hand the setting of the second impulse is delayed and on the other hand the conduction is slowed down. The recent discovery by Blair and Erlanger (1933) of a latent period on the response of a nerve to short induction shocks could be thought as of support of Forbes, Ray and Griffith's assumption; but Professor Erlanger has kindly pointed out to me that the experiments of Blair and Erlanger are not comparable with those of Gasser and Erlanger (1925) in which the shocks were made strong enough to set up the nerve impulse without appreciable latency.

In the case of the nerve muscle preparation the problem seems to be somewhat different because the impulse passes from a tissue of low refractory period (the nerve) to another tissue with long refractoriness (the muscle). It is possible that the endplate itself reacts slower but perhaps a sufficient explanation will be found in the fact that the second impulse is subnormal in size and therefore needs a longer utilization period.

It does not seem necessary to accept for the motoneurones (synapses, dendrites and body) anything fundamentally different from the process happening in the neuromuscular junction.

It has been found that 0.6σ is the upper limit of the absolutely refractory period of the perikaryon, but it has to be left open whether this really

measures the refractory period of the neurone body rather than that of the afferent fibres themselves. Likewise the question of the relative refractoriness of the neurone has to be left open because the delay of the second impulse (0.25 to 0.30σ) might have happened during the setting up of the impulse in the partially refractory axon. From other points of view this problem has been discussed in another paper (Lorente de Nó, 1934).

CONCLUSIONS

The absolutely refractory period of the motoneurones (dendrites and body including the synapses) can not be longer than 0.6σ which is the absolutely refractory period of the stimulated fibres themselves.

The present evidence neither excludes nor proves the existence of a relatively refractory period of the neurone body.

It is suggested that the perikaryon functions in the same way as the muscle endplate, the differences being due to the fact that the endplate receives only one fibre while the neurone receives many terminal fibres.

REFERENCES

Blair, E. A. and J. Erlanger. This Journal **106**: 524, 1933.
Eccles, J. C. Proc. Roy. Soc. *B*.**107**: 557, 1931.
Forbes, A., L H. Ray and F. R. Griffith. This Journal **66**: 553, 1923.
Gasser, H. and J. Erlanger. This Journal **83**: 613, 1925.
Gotch, F. J. Physiol. **40**: 210, 1910.
Lorente, de Nó, R. This Journal, **111**: 272, 1935a. J. f. Psychol. u. Neurol. **46**: 113, 1934. Facilitation and inhibition of motoneurones. To be published. 1935b.
Lucas, K. J. Physiol. **41**: 368, 1910.

SUGAR UTILIZATION IN EVISCERATED RABBITS

D. R. DRURY

From the Collis P. Huntington Memorial Hospital of Harvard University, Boston

Received for publication November 26, 1934

The eviscerated animal is a preparation well suited to the study of sugar utilization. The measurement of the glucose need of the tissues of the intact animal is rendered difficult by the liver, which may be constantly forming this sugar (1); by the pancreas, the internal secretion of which has such a profound effect on the disposal of glucose; and by the intestinal tract which, if it contains food, adds sugar by absorption to the circulating blood. If these organs are removed, we have a preparation in which the sugar utilization of the remaining tissues can be measured. In the work to be reported, this was done by determining the rate that glucose must be supplied to the animal to maintain a normal blood sugar level.

EXPERIMENTAL. Evisceration was carried out by the method of Mann (2) making use of a preliminary partial ligation of the vena cava which produces collaterals sufficient to take care of the circulation when this vessel is tied off. By this method, liver, intestines, spleen and pancreas are removed. In the first animals operated upon saliva accumulated in the obstructed esophagus and was drawn into the lungs, resulting in death. Thereafter to avoid this complication esophageal fistulae were made at the time of operation. Intravenous injection of glucose solution was begun about two hours after operation to maintain the glucose requirement, since these animals, just as hepatectomized ones (3), will become hypoglycemic if not given glucose. It was not necessary to inject glucose immediately after operation since the blood sugar level at that time was very high, due to the anesthetic, and it was best to allow it to come down to a normal level. At frequent intervals blood sugar determinations served as a guide to the minimal amount of glucose capable of maintaining the concentration of blood sugar at approximately 100 mgm. per cent. Throughout the experiments the animals were continuously watched and remained quiet on a warm pad surrounded by a housing to keep them at a normal temperature.

The animals usually sat up within an hour after the anesthetic was withdrawn and appeared normal, sniffing at their surroundings as rabbits do under ordinary circumstances. Urine was secreted in rather large volume at first, although the animals usually became anuric about 24 hours after

operation. Soon after this they began to show weakness and after 27 to 40 hours in all no longer maintained the sitting posture, but lay quietly on the side until death. The animals did not show the clinical symptoms seen in hepatectomized rabbits during the "second stage" (4). There was no period of excitement followed by apparent blindness and ataxia. So long as they could move about they did so quietly and in a normal manner.

Figure 1 illustrates the course of the blood sugar level, and of the glucose injected, in a typical experiment. The sugar requirement is almost constant throughout, and the amount needed is low. Figure 2 illustrates the course of an animal which needed a large amount of sugar at first. It may

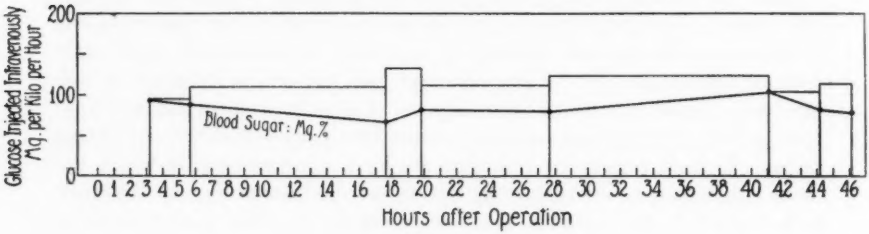


Fig. 1

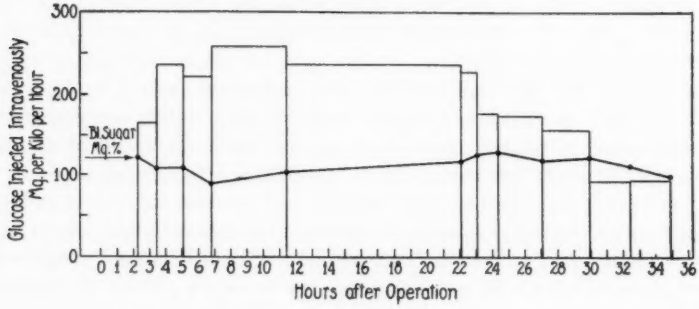


Fig. 2

be noted here (between the second and the seventh hours) that the blood sugar level drops due to an inadequate supply of glucose. Also to be noted is the decrease in sugar requirement after the twenty-second hour.

In table 1 are recorded the survival times, and the glucose utilization rates during the first ten hours after evisceration. In some cases the utilization rate was determined through to the day after operation, and the amounts needed then are recorded in the fifth column. The results indicate a greater need for sugar in those fed up to operation, than in those fasted three days or more before operation, although the requirements tend to become the same after 24 hours. The table shows that in the uncom-

plicated cases, the survival time is not related to the degree of pre-operative fast: animals fed up to the time of operation survive about as long as those given a long fast before operation.

The eviscerated animal seems to be using up glucose at a definite rate. The blood sugar level drops if it is not supplied at this rate. Some of the first animals worked on were actually lost as a result of hypoglycemic convulsions. On the other hand, if glucose be supplied at a rate higher than it is utilized, the blood sugar level rises, and in the first animals high blood sugar levels and even glycosuria were sometimes encountered. With the experience gained with such animals it was possible, in later work, to avoid

TABLE 1

1	2	3	4	5	REMARKS
1	0	27	148		
2	0	35	236	94	
3	0	24	218		Bled considerably
4	0	33	207		
5	0	34			
6	2	33	159	170	
7	2	32	158	149	
8	2	12	142		Died of hypoglycemia
9	2	46	98		
10	3	34	78	110	
11	3	33	70	96	
12	3	35	45	108	
13	3	15	65		Died of pneumothorax
14	4	44	74	118	

Column 1: Animal number.
Column 2: Preoperative fast in days.
Column 3: Survival time in hours.
Column 4: Glucose consumption rate during first 10 hours after operation in milligrams per kilo per hour.
Column 5: Glucose consumption rate about 24 hours after operation in milligrams per kilo per hour.

such occurrences, so that results could be obtained like those depicted in the charts given in this paper, with quite normal blood sugar levels throughout.

Discussion. There are certain noteworthy differences between the results in eviscerated animals and those only hepatectomized, provided adequate glucose be given in every case. The eviscerated rabbit definitely has a longer survival period; the uncomplicated cases practically all live over thirty hours. Hepatectomized rabbits as a rule die in fifteen to thirty hours. Liverless rabbits show a very striking set of symptoms before death (4) similar to those seen in dogs (5). These symptoms are referred to as the "second stage" and consist of irascibility, apparent blindness,

muscular weakness, and ataxia. They are accompanied by a marked increase in the need of the animal for glucose. This syndrome is very characteristic and anyone who has worked with these animals can readily recognize it. No such thing appears in the eviscerated rabbit; the animal shows no unusual symptoms at any time and has no marked increase in need for glucose. These differences in the two types of preparation suggest that the intestinal tract is the cause of the abnormal symptoms of the liverless animal, and give support to the idea that has been frequently advanced that toxic substances are constantly absorbed by the intestine and are rendered innocuous by the liver.

SUMMARY

The rate of glucose utilization by eviscerated rabbits varies between 45 mgm. and 236 mgm. per kilo per hour. Cases without complications survive between 27 hours and 46 hours.

Preoperative fasting seems to cause a decrease in the glucose need, but does not alter the course otherwise.

The definite differences between eviscerated and hepatectomized rabbits are described. These differences support the idea of a detoxifying function of the liver.

REFERENCES

(1) Mann, F. C. J. A. M. A. **85**: 1472, 1925.
(2) Mann, F. C. Ergebn. d. Physiol. **24**: 379, 1925.
(3) Drury, D. R. and P. D. McMaster. Proc. Soc. Exp. Biol. and Med. **27**: 48, 1929.
(4) Drury, D. R. J. Exp. Med. **49**: 759, 1929.
(5) Mann, F. C. Medicine **6**: 419, 1927.

THE INFLUENCE OF DUODENAL SECRETIONS ON ACID GASTRIC CONTENTS

CHARLES M. WILHELMJ, LEO C. HENRICH AND FREDERICK C. HILL

Departments of Physiology and Experimental Surgery, Creighton University School of Medicine, Omaha, Nebraska

Received for publication November 30, 1934

Wilhelmj, Neigus and Hill (1934) studied the composition of duodenal secretions obtained by acid stimulation of isolated duodenal pouches. On repeating these studies on the secretion obtained from the intact non-isolated duodenum (Wilhelmj, Henrich and Hill, 1934) very similar values were obtained for the composition of the mixed duodenal secretions. These studies showed that the alkalinity of the mixed duodenal secretions averages approximately 0.04 normal while the chloride concentration averages approximately 310 mgm. per 100 cc. Using intact normal dogs, it was found (Wilhelmj, Neigus and Hill, 1934) that when tenth normal hydrochloric acid was placed in the stomach and histamin stimulation used, the amount of "extra fluid" which entered the stomach bore a rough relationship to the amount of bile in the gastric sample. From these studies it was concluded that duodenal secretions lower gastric acidity more by dilution than by neutralization and that the neutral chloride present in gastric contents should not be regarded as arising solely from the neutralization of hydrochloric acid, since considerable neutral chloride may enter the stomach as a constituent of the duodenal secretions.

The purpose of the present study was to cause the entrance of large amounts of duodenal secretions into the stomach and to study the effect on acid gastric contents under controlled conditions.

METHODS. Six dogs were used. The general plan was to obtain a series of experiments before and after certain operations designed to cause the entrance of duodenal secretions into the stomach. Each of the following operations was performed on two dogs: Pyloroplasty of the Heinike-Mikulicz type; anterior gastroduodenostomy with wide stoma; anterior gastrojejunostomy with wide stoma. The jejunostomies were made just below the ligament of Trietz. Several weeks were allowed to lapse after the operations before experiments were performed. All animals remained in excellent condition after the operations and were kept in the laboratory for several months, during which time many experiments were performed.

The general procedure in all experiments was to introduce 300 cc. of a

standard hydrochloric acid solution (approximately tenth normal) containing phenol red into the stomach and to allow it to remain for one half-hour; at the end of the half-hour period the stomach was emptied as completely as possible and a second 300 cc. portion of acid phenol red solution introduced, at the end of the second half-hour the stomach was again emptied and a third 300 cc. sample of acid introduced. From three to five half-hour samples were usually obtained during each experiment In some experiments the stomach was stimulated by an intramuscular injection of 1 mgm. of histamin and three half-hour samples obtained after histamin injection, while in other experiments the stomach was not stimulated with histamin. Before starting each experiment the stomach was lavaged with 300 cc. of the acid-phenol red solution to be used in order to remove accumulated secretions and to allow the experiment to start from an approximate zero base line.

The following analyses were made on the samples removed from the stomach: 1. The per cent of phenol red present after the removal of protein, bile pigments and other interfering substances with 10 per cent sodium tungstate and two-thirds normal sulphuric acid. 2. Total and neutral chloride after careful ashing of the thoroughly dried sample. After operation all samples were bile stained but occasionally a large amount of dark green bile was present in the sample, due apparently to emptying of the gall bladder; such samples were usually discarded because of the great difficulty in completely removing the bile before determining the per cent of phenol red.

The general method of calculation was as follows: The reduction in the per cent of phenol red showed the amount of fluid which entered the stomach per 100 cc. of gastric contents, during the half-hour period. When the chloride concentration of the original acid solution was multiplied by the per cent of phenol red in the sample removed from the stomach, the result gave the chloride concentration of the original acid solution corrected for dilution. If the acid chloride in the sample removed from the stomach was above this value the difference represented the amount of acid secreted by the stomach which was not neutralized, while if the acid chloride concentration of the sample removed from the stomach was below the corrected value the difference represented hydrochloric acid which was neutralized and the chloride of this neutralized acid was present with the neutral chloride fraction. The total chloride concentration of the sample removed from the stomach was always above the value of the original acid solution corrected for dilution, and the difference represented the total chloride which was present in the secretions which were mixed with the acid solution while in the stomach. This illustrates the general principles of the calculations. Complete details have been given in the papers by Wilhelmj, Neigus and Hill (1933–1934).

RESULTS. The two animals with pyloroplasties did not show any difference in the amount of duodenal secretions which entered the stomach before and after operation and can be dismissed without further comment. The remaining four dogs all showed evidence of the entrance of large amounts of duodenal secretions into the stomach. The experiments will be considered under two headings. *First*, those in which the stomach was not stimulated with histamin, and *second*, those in which histamin stimulation was used.

A. *Experiments after gastroduodenostomy and gastrojejunostomy without histamin stimulation.* Only those experiments in which there was no evidence of secretion of hydrochloric acid by the stomach will be considered under this heading. A complete set of experiments on two dogs after gastroduodenostomy and two dogs after gastrojejunostomy is given in table 1.

There was no essential difference in the composition of the intestinal fluid which entered the stomach after these two types of operation. This is probably due to the fact that the gastrojejunostomies were made just at the end of the duodenum so that the fluid which entered the stomach was primarily of duodenal origin.

1. *The acid chloride concentration of the samples removed from the stomach* (col. 7) average 231 mgm. per 100 cc. in 23 experiments. The acid chloride concentration of the acid solution introduced into the stomach was approximately 350 mgm. per 100 cc. (col. 2), the average difference of 119 mgm. of acid chloride per 100 cc. represents the total dercease in acid, during the half-hour period, and is the combined result of both neutralization and dilution.

2. *The chloride of acid below the level of the original acid solution corrected for dilution* (col. 9) represents the reduction in acid chloride due to neutralization, this averages 31 mgm. of chloride per 100 cc.

3. *The alkalinity of the duodenal secretions entering the stomach* (col. 13) averaged 0.04 normal with variations ranging between 0.01 and 0.07 normal. Previous studies on duodenal secretions reported by Wilhelmj, Neigus and Hill (1934) and by Wilhelmj, Henrich and Hill (1934) gave the same average value with approximately the same variations. From the data given in table 1 it is possible to determine the relative importance of dilution and neutralization in lowering the acidity of approximately tenth normal hydrochloric acid solutions by duodenal secretions. This is graphically illustrated in figure 1 where it is seen that on the average 75 per cent of the reduction in acidity was due to dilution and 25 per cent due to neutralization. The data, from the experiments reported by Wilhelmj, Neigus and Hill (1934) in which approximately tenth normal hydrochloric acid was instilled into isolated duodenal pouches, have been recalculated in a similar manner and it was found that an average of 68 per cent of the

total reduction in acid was due to dilution and 32 per cent due to neutralization. In secretions obtained from the intact duodenum, stimulated with tenth normal hydrochloric acid, Wilhelmj, Henrich and Hill (1934) found

TABLE 1

Complete data from 23 experiments on two dogs after gastroduodenostomy and two dogs after gastrojejunostomy

Three hundred cubic centimeters of approximately tenth normal hydrochloric acid containing phenol red were placed in the stomach and allowed to remain for one-half hour. Stomach not stimulated with histamin. Calculations show the amount and composition of the duodenal secretions entering the stomach.

DOG	ACID SOLUTION USED, MGM. ACID CHLORIDE PER 100 CC.	P.S.P., PER CENT	MGM. PER 100 CC.							MGM. NEUTRALIZED ACID CHLORIDE / CC. OF SECRETION	NEUTRAL CHLORIDE / CC. OF SECRETION	NORMALITY OF SECRETION	CC. OF SECRETION IN TOTAL VOLUME	VOLUME, CC.
			Chloride of original acid solution corrected for dilution	Total chloride	Neutral chloride	Total minus neutral chloride	Extra chloride	Chloride of acid below correction for dilution	Chloride concentrations of secretions entering stomach					
I. Gastroduodenostomy	348	75	261	340	134	206	79	55	316	2.2	5.4 (3.8)	0.06	87	346
		78	272	340	119	221	68	51	309	2.3	5.4 (3.8)	0.06	62	283
		79	275	345	116	229	70	46	333	2.2	5.5 (4.0)	0.06	65	310
I. Gastroduodenostomy	351	78	274	351	94	257	77	17	350	0.8	4.3 (3.8)	0.02	59	266
		76	267	348	138	210	81	57	338	2.4	5.7 (4.1)	0.07	83	346
		77	270	348	122	226	78	44	339	1.9	5.3 (3.9)	0.05	58	252
II. Gastroduodenostomy	348	68	237	336	111	225	99	12	310	0.4	3.5 (3.3)	0.01	82	256
		67	234	336	118	218	102	16	310	0.5	3.6 (3.3)	0.01	101	306
		69	240	334	103	231	94	9	304	0.3	3.3 (3.1)	0.01	88	284
II. Gastroduodenostomy	351	65	228	330	127	203	102	25	291	0.7	3.6 (3.3)	0.02	102	290
		69	242	337	116	221	95	21	307	0.7	3.7 (3.3)	0.02	51	165
		73	256	333	111	222	77	34	286	1.3	4.1 (3.4)	0.04	73	270
Average						226		32	316	1.3	4.5 (3.6)	0.04	76	
III. Gastrojejunostomy	349	74	258	342	114	228	84	30	323	1.2	4.4 (3.7)	0.03	85	328
		77	269	336	109	227	67	42	292	1.8	4.7 (3.6)	0.05	67	292
		81	282	342	100	242	60	40	316	2.1	5.3 (3.8)	0.06	60	314
III. Gastrojejunostomy	349	78	272	342	87	255	70	17	318	0.8	4.0 (3.5)	0.02	70	320
		77	269	340	111	229	71	40	309	1.7	4.8 (3.7)	0.05	71	310
		82	286	342	102	240	56	46	311	2.4	5.4 (3.8)	0.07	64	338
IV. Gastrojejunostomy	351	71	250	348	147	201	98	49	338	1.7	5.1 (4.0)	0.05	90	310
		81	284	345	89	256	61	28	321	1.5	4.7 (3.7)	0.04	55	290
IV. Gastrojejunostomy	351	61	214	351	142	209	137	5	351	0.13	3.6 (3.6)	0.004	129	330
		72	253	343	98	245	90	8	322	0.29	3.5 (3.4)	0.01	74	265
		81	284	352	80	272	68	12	358	0.63	4.2 (3.8)	0.02	61	320
Average						234		29	324	1.3	4.5 (3.7)	0.04	75	
Average for 23 experiments						231		31	320	1.3	4.5 (3.6)	0.04	76	

that 73 per cent of the reduction in acid was due to dilution and 27 per cent to neutralization. These three groups of data are in excellent agreement and show that, on the average, approximately 75 per cent of the reduction

of the acidity of tenth normal hydrochloric acid by duodenal secretions is due to dilution and only 25 per cent due to neutralization.

4. *The amount of fluid entering the stomach per 100 cc. of gastric contents during the half-hour period* is shown by the reduction in the per cent of phenol red (col. 3); when this is multiplied by the volume of the sample, the result gives the total amount of fluid in the entire sample (col. 14). The average total amount per half-hour was 76 cc. with maximal and minimal amounts of 129 and 51 cc. in individual experiments. In studies on

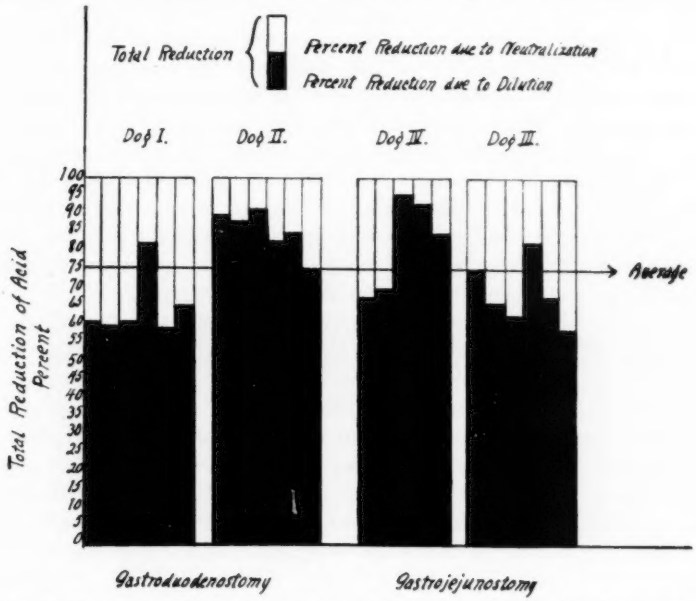


Fig. 1. Shows the relative importance of dilution and neutralization in lowering the acidity of approximately tenth-normal hydrochloric acid placed in the stomach after gastroduodenostomy and gastrojejunostomy. Stomach not stimulated with histamin. Dog III had complete anacidity even after histamin stimulation at the time that these experiments were performed.

whole stomach pouches under similar conditions Wilhelmj, Henrich and Hill (1934) found that the total amount of non-acid fluid of intragastric origin averaged only 8 cc. per half-hour with maximal and minimal values of 14 and 4 cc. This comparison shows that most of the fluid entering the stomach in the present experiments was of duodenal origin.

5. *The chloride concentration of the fluid entering the stomach* (col. 10) averaged 320 mgm. per 100 cc. and ranged from 286 to 358 mgm. per 100 cc. in individual experiments. In experiments on isolated duodenal

pouches Wilhelmj, Neigus and Hill (1934) found the chloride concentration of duodenal secretions obtained by acid stimulation to average 310 mgm. per 100 cc. with approximately the same maximal and minimal values.

6. *The ratio of neutral chloride to the cubic centimeters of secretion* (col. 12) shows the amount of neutral chloride related to each cubic centimeter of secretion entering the stomach and includes the neutral chloride content of each cubic centimeter plus the neutral chloride arising from the neutralization of hydrochloric acid by each cubic centimeter of secretion. This ratio averages 4.5 and ranges between 3.3 and 5.7 in individual experiments. In order to make this ratio more nearly comparable to that obtained when the stomach is secreting hydrochloric acid, some of which is neutralized by the duodenal secretions, we have corrected it by assuming that the acid neutralized contained 578 mgm. of chloride per 100 cc. instead of 350 mgm. per 100 cc. and adding the fluid of this neutralized acid to the cubic centimeter of duodenal secretion; the corrected ratios are given in parenthesis and average 3.6 for the 23 experiments. Wilhelmj, Neigus and Hill (1934) found non-corrected and corrected ratios of 4.7 and 3.7 respectively in duodenal secretions from isolated duodenal pouches. The importance of this ratio will be discussed more in detail in the next section.

The calculation of the composition of the duodenal secretions from the experiments given in table 1 could be criticized by stating that small amounts of hydrochloric acid were secreted by the stomach and neutralized by the duodenal secretions, so that the calculated alkalinity of the duodenal secretions would be lower than the true value while the chloride concentration would be higher. However, this criticism does not appear to be valid for the following reasons: *First*, dog III, for some unknown reason, developed an almost complete anacidity after performing the gastrojejunostomy. This anacidity was so complete that there was no secretion of acid even after the intramuscular injection of 1 mgm. of histamin (fig. 2). In spite of this the general condition of the animal was very satisfactory. This anacidity lasted for approximately two months and during this period the experiments shown in table 1 were performed. It is seen that the composition of the duodenal secretions in this dog is not essentially different from that of the other three dogs. Second, the composition of the duodenal secretions entering the stomach as shown in table 1, is in excellent agreement with that found by Wilhelmj, Neigus and Hill (1934) for duodenal secretions obtained by acid stimulation of isolated duodenal pouches. These considerations make it rather improbable that sufficient hydrochloric acid was being secreted by the non-stimulated stomach to significantly alter the composition of the duodenal secretions entering the stomach.

B. *Experiments before and after gastroduodenostomy and gastrojejunostomy with histamin stimulation* A series of experiments before and after gastroduodenostomy (dog 1) and gastrojejunostomy (dog IV) is given in detail in table 2.

The following points deserve special comment: 1. *The amount of fluid entering the stomach per 100 cc. of gastric contents* is obtained by subtracting the per cent of P.S.P. in the gastric sample (col. 4) from 100. It is quite evident that more fluid entered the stomach after operation than before, and in the experiments shown in table 2, the gastroduodenostomy appears to be somewhat more efficient in this respect than the gastrojejunostomy. Wilhelmj, Neigus and Hill (1934) have shown that, because of the approximate constancy of the chloride concentration of fundic secretion, it is

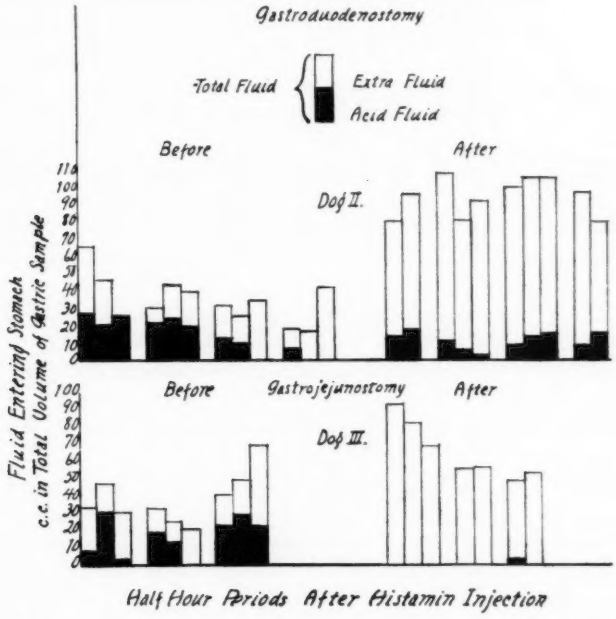


Fig. 2. Shows the total, acid and extra fluid entering the stomach before and after gastroduodenostomy and gastrojejunostomy. Results expressed as the *total quantities* in the entire gastric sample. Stomach stimulated with intramuscular injection of 1 mgm. histamin. Note the anacidity in dog III after operation.

possible to separate the total fluid which enters the secreting stomach into two fractions, first, the fluid of hydrochloric acid which was secreted by the stomach and not neutralized and second, the "extra fluid" which consists of pyloric secretions, fundic mucus, duodenal secretions and the fluid of acid which was secreted by the stomach and subsequently neutralized. This calculation is made by dividing the chloride of hydrochloric acid which was secreted and not neutralized (chloride of acid above the corrected level (col. 10)) by 5.78. This gives the cubic centimeters of acid fluid and

TABLE 2

Experiments before and after gastroduodenostomy and gastrojejunostomy

Three hundred cubic centimeters of approximately tenth normal hydrochloric acid containing phenol red were placed in the stomach and allowed to remain for one hour. Stomach stimulated with one milligram of histamin intramuscularly.

DOG	ACID SOLUTION USED, MGM. CHLORIDE PER 100 CC.	TIME AFTER HISTAMIN	P.S.P., PER CENT	MGM. PER 100 CC.						CC. OF ACID FLUID	CC. OF EXTRA FLUID	CC. OF TOTAL FLUID	NEUTRAL CHLORIDE / CC. EXTRA FLUID	CC. EXTRA FLUID IN TOTAL VOLUME	VOLUME OF SAMPLE	REMARKS
				Chloride of original acid solution corrected for dilution	Total chloride, mgm.	Neutral chloride, mgm.	Total minus neutral chloride, mgm.	Extra chloride, mgm.	Chloride of acid ± corrected value	Chloride concentration of secretion, mgm.						
I. Before gastroduodenostomy	348	½	85	296	376	30	346	80	+50	533	9	6	15	5.0	18	306	
		1	85	296	379	24	355	83	+59	555	10	5	15	4.8	14	274	
		1½	88	306	348	67	281	42	−25	350	0	12	12	(4.2)*	—	50	Vomited
I. Before gastroduodenostomy	348	½	83	289	370	44	326	81	+37	476	6	11	17	4.0	33	298	
		1	83	289	380	42	338	91	+49	534	8	9	17	4.7	25	278	
		1½	89	310	370	46	324	60	+14	546	2	9	11	5.1	23	258	
I. Before gastroduodenostomy	348	½	87	303	366	22	344	63	+41	485	7	6	13	3.7	22	372	
		1	85	296	378	26	352	82	+56	546	10	5	15	5.2	15	306	
		1½	94	327	354	33	321	27	−6	450	0	6	6	(4.7)*	14	232	
Average											5.8	7.7	13.5	4.6	21		
I. After gastroduodenostomy	345	½	66	228	354	94	260	126	+32	371	6	28	34	3.4	78	280	
		1	55	190	348	108	240	158	+50	351	9	36	45	3.0	109	301	
		1½	61	210	345	107	238	135	+28	346	5	34	39	3.2	93	274	
I. After gastroduodenostomy	345	½	50	173	397	107	290	224	+117	447	20	30	50	3.6	105	349	
		1	61	210	357	143	214	147	+4	377	0.7	38.3	39	3.7	93	242	
		1½	64	221	346	129	217	125	−4	348	0	36	36	(3.5)*	73	203	
I. After gastroduodenostomy	345	½	61	210	390	105	285	180	+75	463	13	26	39	4.0	63	240	
		1	64	221	378	118	260	157	+39	437	7	29	36	4.1	128	440	
		1½	70	242	333	125	208	91	−34	303	0	30	30	(3.5)*	82	274	
I. After gastroduodenostomy	345	½	59	204	379	125	254	175	+50	427	9	32	41	3.9	97	302	
		1	60	207	379	93	286	172	+79	429	14	26	40	3.6	80	308	
		1½	69	238	354	108	246	116	+8	274	1.4	29.6	31	3.7	85	288	
Average											7.1	31.2	38.3	3.6	91		
IV. Before gastrojejunostomy	353	½	89	314	372	31	341	58	+27	528	5	6	11	5.2	18	306	
		1	83	293	390	30	360	97	+67	570	12	5	17	6.0	16	318	
		1½	92	325	372	26	346	47	+21	587	4	4	8	6.5	12	296	
IV. Before gastrojejunostomy	353	½	85	300	367	48	319	67	+19	447	3	12	15	4.0	28	233	
		1	86	304	375	39	336	71	+32	507	6	8	14	4.9	27	332	
		1½	90	318	366	26	340	48	+22	480	4	6	10	4.3	15	246	
IV. Before gastrojejunostomy	353	½	89	314	372	30	342	58	+28	527	5	6	11	5.0	19	310	
		1	93	328	366	19	347	38	+19	544	3	4	7	4.8	12	311	
		1½	94	332	366	24	342	34	+10	567	1.7	4.3	6	5.6	11	256	
Average											4.9	6.1	11	5.1	18		
IV. After gastrojejunostomy	352	½	76	268	373	73	300	105	+32	438	6	18	24	4.1	57	316	
		1	85	299	375	44	331	76	+32	507	6	9	15	4.9	22	246	
		1½	81	285	364	84	280	79	−5	416	0	19	19	(4.2)*	58	306	
IV. After gastrojejunostomy	348	½	74	258	366	84	282	108	+24	415	4	22	26	3.8	73	330	
		1	72	250	360	95	265	110	+15	393	3	25	28	3.8	83	330	
		1½	79	275	348	91	257	73	−18	348	0	21	21	(3.8)*	66	316	
IV. After gastrojejunostomy	348	½	73	254	372	89	283	118	+29	437	5	22	27	4.0	81	368	
		1	77	268	376	67	309	108	+41	470	7	16	23	4.2	43	270	
		1½	84	292	354	80	274	62	−18	388	0	16	16	(4.2)*	45	280	
Average											3.4	18.7	22.1	4.1	59		

* When an acid deficit occurred the neutral chloride extra fluid ratio was calculated from the extra neutral chloride and not from the total.

the difference between this and the total fluid entering the stomach represents the "extra fluid." The value of 578 mgm. per 100 cc. for the chloride concentration of fundic secretion is that obtained by Wilhelmj, Neigus and Hill (1933). The recent studies of Hollander show that pure parietal secretion probably contains no neutral chloride and that the chloride concentration of the hydrochloric acid as secreted is approximately 600 mgm. per 100 cc., it would therefore probably be more accurate to divide the chloride of secreted acid by 6.0 than by 5.78. However, this correction would introduce only a negligible change in our values.

In columns 12, 13 and 14 the amounts of acid, extra and total fluid per 100 cc. of gastric sample are shown.

2. *The "extra fluid" entering the stomach per 100 cc. of gastric contents before and after operation* is shown in column 13. In dog I before operation the average amount was 7.7 cc. per 100 cc. of gastric contents, while after gastroduodenostomy the average amount increased to 31.2 cc. per 100 cc. In dog IV before and after gastrojejunostomy the corresponding average values were 6.1 and 18.7 cc. respectively. When the amount of extra fluid per 100 cc. is multiplied by the volume of the sample removed from the stomach the result gives the amount of extra fluid in the entire sample. This is shown in column 16. Before gastroduodenostomy the *total amount* of extra fluid in the sample averaged 21 cc., while after operation the average quantity was 91 cc. Before and after gastrojejunostomy the corresponding values were 18 cc. and 59 cc. respectively. It should be remembered that the *total amount* of "extra fluid" in the sample removed from the stomach is less than the actual total amount of duodenal fluid which entered the stomach during the half-hour period because a certain unknown amount of the gastric contents left the stomach during the half-hour period. The studies of Wilhelmj, Henrich and Hill (1934) on whole stomach pouches investigated under identical conditions and with histamin stimulation showed that the total amount of "extra fluid" of intragastric origin averaged only 6 cc. per half-hour and varied from 2 to 13 cc. in individual experiments. Hence in the experiments shown in table 2 most of the extra fluid is of duodenal origin.

3. *The neutral chloride-extra fluid ratio* (col. 15) shows the amount of neutral chloride related to each cubic centimeter of extra fluid and includes the neutral chloride content of each cubic centimeter plus the neutral chloride resulting from the neutralization of hydrochloric acid by each cubic centimeter of extra fluid. In dog I before gastroduodenostomy the average ratio was 4.6 while after operation the average value was 3.6. In dog II before and after gastrojejunostomy the average ratios were 5.1 and 4.1 respectively. Wilhelmj, Neigus and Hill (1934) called attention to the fact that this ratio is often slightly higher in the extra fluid of intragastric origin than in that of duodenal origin and that in normal dogs the ratio frequently drops as duodenal fluid regurgitates into the stomach.

Theoretically the "extra fluid" entering the stomach in the experiments in which histamin stimulation was used should be identical with the non-acid fluid which entered the stomach in the experiments without histamin stimulation (table 1). The following findings suggest the identity: *First*, the neutral chloride extra fluid ratios, after operation, in experiments with histamin stimulation, are practically the same as the ratio of the neutral chloride to the cubic centimeters of fluid without histamin stimulation; *second*, the total amount of "extra fluid" in the experiments with histamin stimulation after operation averages 75 cc. for 21 experiments (table 2)

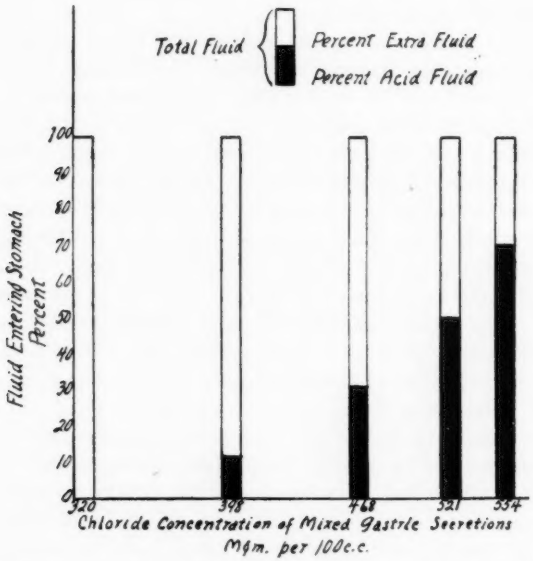


Fig. 3. An analysis of 84 half-hour samples before and after gastroduodenostomy showing that the chloride concentration of the mixed secretions entering the stomach depends upon the relative amounts of acid and "extra fluid."

while the total amount of fluid entering the stomach, after operation, in 23 experiments without histamin stimulation averages 76 cc. (table 1).

4. *The fluid of acid which was secreted and not neutralized* (col. 12) is calculated directly from the acid chloride above the corrected value, shown in column 10. It is seen that there is relatively little difference in the values before and after operation. This is also graphically illustrated in figure 2 (dog II). This is a rather surprising and confusing finding, for if we grant an average alkalinity of 0.04 normal for the large amount of duodenal fluid which entered the stomach after operation, then more of the

secreted acid should be neutralized, hence less should be present after operation. The finding of approximately the same amount can only mean that more acid was secreted by the stomach when stimulated with histamin after operation. There was no evidence of a spontaneous, continuous hypersecretion of acid after operation for, as seen in table 1, the non-stimulated stomach did not secrete acid when simply filled with tenth normal hydrochloric acid solution. The hypersecretion seemed to occur only after secretion had been started by histamin stimulation. It is quite possible that the explanation of this phenomenon rests upon the observation of Meyer, Ivy and McEnery (1924). They found that when bile was introduced into the main stomach of Pavlov pouch dogs it definitely stimulated secretion which was most marked after the pouch had been stimulated to secrete, or as they state, "This is especially true if the glands are in tone, i.e., secreting free acid, at the time the bile is given." Our observation is a very interesting one and may be a factor of considerable importance if it occurs when gastrojejunostomy is done for relief of peptic ulcer.

5. *The chloride concentration of the mixed secretions entering the stomach* will depend upon the *relative* (not the absolute) amounts of acid fluid which has a chloride concentration of from 578 to 600 mgm. chloride per 100 cc. and "extra fluid" which has a chloride concentration of approximately 320 mgm. per 100 cc. This is shown graphically in figure 3. This figure should be compared with a similar one in the article by Wilhelmj, Henrich and Hill (1934) made from data on whole stomach pouches. This similarity is due to the fact that the extra fluid of intragastric origin has approximately the same chloride concentration as the extra fluid of duodenal origin.

6. *The total volume of gastric sample removed each half-hour* (col. 17) was approximately the same before and after operation. The similarity of the volumes of the samples justifies the comparison of the amounts of extra, acid and total fluid in the total volume of the sample removed before and after operation (fig. 2).

SUMMARY

1. The influence of duodenal secretions on acid gastric contents was studied in six dogs before and after pyloroplasty, gastroduodenostomy and gastrojejunostomy. In two animals after pyloroplasty there was no evidence that more duodenal secretions entered the stomach than before operation. In two animals after gastroduodenostomy and two after gastrojejunostomy, large amounts of duodenal secretions entered the stomach.

2. The *total amount* of duodenal secretions in the samples removed from the stomach after operation averaged 75 cc. per half-hour and occasionally exceeded 100 cc. in individual experiments.

3. The average composition of the duodenal secretions was the same as

that previously found: The average alkalinity being approximately 0.04 normal while the chloride concentration averaged 320 mgm. per 100 cc.

4. The efficiency of duodenal secretions in lowering the acidity of approximately tenth normal hydrochloric acid in the stomach is due more to dilution than to neutralization, on the average 75 per cent of the reduction in acidity was due to dilution and 25 per cent to neutralization.

5. The presence of large amounts of duodenal secretions in the stomach seems to cause a hypersecretion of acid by the stomach which is evident only after the stomach has been stimulated to secrete by histamin and not in the non-stimulated stomach.

6. The chloride concentration of the mixed gastric secretions depends upon the relative amounts of acid fluid and extra fluid present.

REFERENCES

(1) Hollander, F. J. Biol. Chem. **97**: 585, 1932.
This Journal **98**: 551, 1932.
Proc. Soc. Exp. Biol. and Med. **29**: 640, 1932.
J. Biol. Chem. **104**: 33, 1934.
(2) Meyer, J., A. C. Ivy and E. T. McEnery. Arch. Int. Med. **34**: 129, 1924.
(3) Wilhelmj, C. M., I. Neigus and F. C. Hill. This Journal **106**: 381, 1933.
(4) Wilhelmj, C. M., I. Neigus and F. C. Hill. This Journal **107**: 490, 1934.
(5) Wilhelmj, C. M., L. C. Henrich and F. C. Hill. Proc. Soc. Exp. Biol. and Med. **31**: 969, 1934.
(6) Wilhelmj, C. M., L. C. Henrich and F. C. Hill. This Journal **110**: 251, 1934.

SODIUM, CHLORIDE AND PROTEIN CHANGES INDUCED BY ADRENALECTOMY AND GLUCOSE ADMINISTRATION

H. SILVETTE[1] AND S. W. BRITTON

From the Physiological Laboratory of the University of Virginia Medical School

Received for publication November 27, 1934

It is well known that the conditions of water balance in the body cannot in the present state of physiological and biochemical knowledge be adequately described. According to Adolph (1933), "even for blood plasma only part of the factors entering the equilibrium have been correlated." This statement is true also with respect to ionic relationships in the tissues and body fluids. One constituent cannot be considered by itself, and even though many may be studied at once, still other factors remain obscure and preclude dogmatic generalization. Certain chemical substances in the blood and tissues appear nevertheless to play a major rôle in bodily equilibria and are particularly deserving of study. Implications which have been put forward in a number of recent reports (Loeb et al., 1933; Harrop et al., 1933; Zwemer and Sullivan, 1934) that the adrenal cortex is particularly involved in salt and water metabolism, connected as they possibly are with our own carbohydrate theory of cortico-adrenal function (Britton and Silvette, 1932, 1934), have furthermore stimulated interest in this subject.

In an attempt to correlate as many factors as appeared feasible in a consideration of salt and water metabolism following bilateral adrenalectomy, analyses for serum sodium, chlorides, protein and glucose have been performed in several extended series of animals. In addition, data were obtained from hepatic and muscle tissue on the salt and water concentrations found therein in normal and adrenalectomized animals, and in animals subjected to various experimental conditions.

METHODS. The procedures for the determination of serum and tissue water, chlorides and carbohydrates have been previously described (Silvette and Britton, 1932, 1933; Silvette, 1934). Sodium was determined by the uranyl-zinc-sodium acetate method of Butler and Tuthill (Peters and Van Slyke, 1932); serum protein by the gravimetric method of Guillamin, Wahl and Laurencin (Peters and Van Slyke, 1932). Cats were used for all the observations; they were fasted for 18 hours previous to the beginning of an experiment. Several series of animals were bilaterally ad-

[1] E. R. Squibb and Sons Scientific Fellow in Physiology.

renalectomized in a one-stage operation. Other experimental procedures will be indicated later.

RESULTS. *Normal animals.* Analyses of blood serum and muscle and liver of normal 18-hour-fasted cats compared favorably with those previously reported from this Laboratory and by other workers (Katz, 1896; Baumann and Kurland, 1927; Lang, 1931; Silvette, 1934). To conserve space, average figures and variations only are given in table 1. Statistical treatment of the serum sodium and chloride figures in the several series appears in figure 1.

TABLE 1

Sodium, chloride, protein and water content of serum and tissues of 13 normal cats, fasted 18 hours

	WATER		SODIUM			CHLORIDES			SERUM PROTEIN	SERUM SUGAR
	Muscle	Liver	Serum	Muscle	Liver	Serum	Muscle	Liver		
	per cent	*per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*per cent*	*mgm. per cent*
Maximum.....	77.2	73.8	371	81	169	456	54	114	8.4	100
Minimum.....	74.5	62.4	348	60	91	428	38	85	5.9	73
Average.....	75.7	69.5	358	71	130	439	48	102	6.9	87

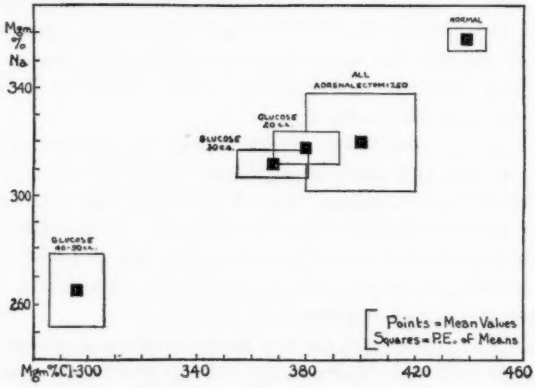


Fig. 1

Adrenalectomy. After adrenal removal there occurred a progressive loss of serum and tissue sodium and chlorides, and a slight diminution in serum protein (table 2, A and B). There seemed to be no correlation, however, between the increase in muscle water observed and the lowered serum protein level. The loss of salt from blood and tissues and the increase in tissue water content possibly lead in the adrenalectomized cat to a

state of subnormal osmotic pressure in the body tissues and fluids. This problem is now being further investigated.

Adrenalectomy and fasting. Adrenalless animals fasted for 4 to 6 days following the operation showed very low sodium and chloride levels throughout the body (table 2 C). Serum protein was also considerably reduced. The data indicated that the salt loss observed after adrenalectomy was aggravated by the salt deprivation of starvation. Thus, lower serum sodium and chloride levels were found in animals fasted and surviving 4 to 6 days after adrenalectomy than were found in simple long-term survival cases. It should be emphasized that normal animals deprived of food for 7 days showed no deviations from the normal serum sodium, chloride and protein levels:

CAT	MGM. PER CENT SERUM					
	Initial samples			After 7-day fast		
	Na	Cl	Protein	Na	Cl	Protein
A	360	438	7000	362	445	6800
B	366	455	7000	357	444	7000
C	354	450	6200	351	441	6100

Glucose administration. Intraperitoneal injections of isotonic or hypertonic glucose brought about a diminution of serum sodium and chloride as severe as, and on the average more severe than, that observed after adrenal removal. Muscle and liver sodium chloride was reduced to similar low levels. In such experiments the water balance, in contrast to the salt balance, was not appreciably disturbed (table 3).

When large amounts of glucose were injected (table 3 C), the animals later showed definite intoxication. The condition was probably referable to extreme salt depletion of the blood and tissues or possibly to the very high blood-glucose concentrations. Such animals showed frequent fine muscular twitchings or tremors which were obviously quite different in character from the severe convulsions arising from blood sugar reduction after adrenal removal or insulin injection.

It may be observed from figure 1 that after adrenalectomy there are reductions from the normal serum sodium and chloride levels of 11 per cent and 9 per cent respectively. In contrast to this, the sodium and chloride levels after glucose injection fell as low as 26 per cent and 33 per cent respectively before death supervened. Reductions of 13 per cent and 17 per cent after glucose administration did not appear to disturb the general condition of the animal.

The observation of Gilman (1934) that withdrawal of blood from salt-depleted dogs may lead to a fatal result has been confirmed in the case of

cats whose serum sodium and chloride at the time of bleeding was critically low (see fig. 2). The cause of death in these instances appeared to be due to asphyxia which supervened on reducing the blood volume, already disturbed by the excretion of water and salts *via* the kidney and peritoneum. Again, however, the possible untoward effects of large amounts of glucose suddenly introduced into the blood and tissues is to be kept in mind.

TABLE 2

Tissue sodium and chloride concentrations and serum protein following adrenalectomy

	SURVIVAL	WATER		SODIUM			CHLORIDES			SERUM PROTEIN	SERUM SUGAR
		Muscle	Liver	Serum	Muscle	Liver	Serum	Muscle	Liver		
A. Short-surviving cats (9 cases)											
	days	*per cent*	*per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*per cent*	*mgm. per cent*
Maximum	3	78.2	74.9	371	69	170	453	55	139	9.0	65
Minimum	2	75.1	64.4	296	44	72	371	34	79	5.6	35
Average	2.7	76.6	71.4	340	54	125	418	40	110	6.9	54
B. Long-surviving cats (9 cases)											
Maximum	14	78.7	75.2	324	68	148	427	45	110	8.4	63
Minimum	4	76.0	68.4	267	20	45	340	33	72	4.5	50
Average	8.7	77.4	73.1	306	45	95	388	38	89	6.7	57
C. Cats fasted after adrenal removal (5 cases)											
Maximum	6	78.1	75.3	327	65	144	410	51	132	7.2	74
Minimum	4	76.4	71.0	274	44	47	370	32	72	5.1	40
Average	5.2	76.9	73.5	298	56	110	387	41	104	6.3	57

DISCUSSION. It is to be emphasized that after adrenal removal in the cat the blood is the only notably dehydrated tissue, and that the condition is one of hemoconcentration rather than plasma dehydration (Silvette and Britton, 1934). In contrast, sodium and chloride concentrations are reduced not only in the blood serum, but in muscle and liver as well (Hartman, 1934; Silvette, 1934).

In all conditions studied in which salt was lost from the blood serum and tissues of cats, sodium and chloride loss showed a straight-line relationship (figure 1). It was thus interpreted that sodium and chloride did not disappear from the body separately; rather, the neutral salt appeared to be excreted. In this connection it is interesting to note that nervous tissue

immersed in isotonic sugar solution loses sodium and chloride in approximately equal amounts (Fenn et al., 1934).

It thus seems improbable that the adrenal cortex, even though it may be indirectly concerned with salt balance, exerts any specific influence over either sodium or chloride metabolism, the one independently of the other. It is possible that the cortical hormone may be linked with water balance, and that the heightened excretion of sodium (Loeb et al., 1933; Harrop et al., 1933) and chloride (Silvette and Britton, 1933) and the lowered

TABLE 3

Effect of glucose injection on serum sodium and chloride levels

First injection of 10 per cent glucose at 0 hour; second at end of 4th hour; animals sacrificed at end of 6th hour.

	MUSCLE WATER	SODIUM		CHLORIDES		SERUM SUGAR
		Serum	Muscle	Serum	Muscle	
A. 20 cc. per kilo body weight (9 animals used)						
	per cent	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*
Maximum	77.3	327	38	403	48	408
Minimum	75.5	303	25	343	25	278
Average	76.6	318	31	380	34	336
B. 30 cc. per kilo (8 animals)						
Maximum		324		390		328
Minimum		300		346		225
Average		312		366		280
C. 40–50 cc. per kilo (5 animals)*						
Maximum		291		311	22	650
Minimum		231		270	21	230
Average		266		297	22	432

* In 2 cases, 40 cc.; in 3 cases, 50 cc. per kilo.

concentration of these substances found in the tissues of at least some species after adrenalectomy are functions of the need for reduction of the osmotic pressure in the tissues without preponderant increase in water content or excessive sacrifice of protein and carbohydrate material.

The disturbances in salt and water metabolism which follow adrenalectomy in cats are still to be considered as secondary phenomena, from our experiments, in the train of symptoms which are characteristic of adrenal insufficiency. This follows from the observation that reductions in salt

content of body tissues and fluids even greater than those observed after adrenalectomy have no deleterious effect on the otherwise normal animal. It is noteworthy that it is necessary to force the sodium and chloride levels two to three times below those observed in adrenalectomy before a fatal result occurs. Furthermore, symptoms which appear following excessive salt loss are qualitatively different from those observed in adrenal insufficiency. Administration of sodium chloride to adrenalectomized dogs also has only a temporary palliative effect (Swingle et al., 1934; Zwemer, 1934).

In respect to tissue water and salt, and serum sodium, chloride and protein, no critical condition has been observed in our experiments on adrenalectomized cats. The diminutions in blood glucose and liver glycogen remain the only observable chemical changes which, by direct experiment on many different species and also by analogous reasoning, are sufficient to explain the adrenalless animal's death.

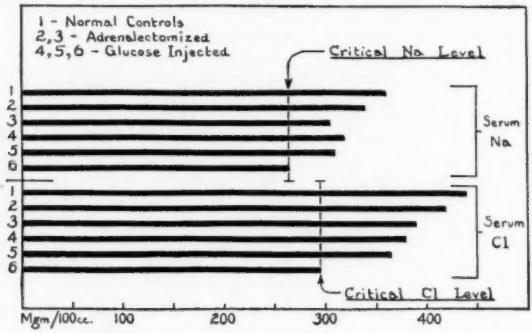


Fig. 2

SUMMARY

After adrenalectomy in cats there occurs a progressive diminution in sodium and chloride concentrations in blood serum and liver and muscle tissues. The serum protein is also somewhat reduced.

Intraperitoneal injection of glucose solution depresses the tissue and serum sodium and chloride concentrations to levels much lower than those observed in adrenal insufficiency, but without deleterious effect on the animal. Extreme reductions of sodium and chloride, two to three times below those found after adrenalectomy, must be imposed before a fatal result ensues.

Muscular tremors and prostration follow the administration of large amounts of glucose by intraperitoneal injection. The condition, which is apparently due to excessive salt loss induced by osmotic interchange, may

last 24 hours or more before ending in recovery or death. It is qualitatively and fundamentally different from the severe convulsive (hypoglycemic) seizures of adrenal insufficiency.

REFERENCES

ADOLPH, E. F. Physiol. Rev. **13:** 336, 1933.
BAUMANN, E. J. AND S. KURLAND. J. Biol. Chem. **71:** 281, 1927.
BRITTON, S. W. AND H. SILVETTE. This Journal **100:** 701, 1932.
Ibid. **107:** 190, 1934.
FENN, W. O. ET AL. This Journal **110:** 74, 1934.
GILMAN, A. This Journal **108:** 662, 1934.
HARROP, G. A. ET AL. J. A. M. A. **100:** 1850, 1933.
J. Exp. Med. **58:** 1, 17, 1933.
HARTMAN, F. A. Ann. Int. Med. **7:** 6, 1933.
KATZ, J. Pflüger's Arch. **63:** 1, 1896.
LANG, K. Pflüger's Arch. **229:** 60, 1931.
LOEB, R. F. ET AL. J. Exp. Med. **57:** 775, 1933.
PETERS, J. P. AND D. D. VAN SLYKE. Quantitative clinical chemistry. Volume II, Methods. Baltimore, 1932.
SILVETTE, H. This Journal **108:** 535, 1934.
SILVETTE, H. AND S. W. BRITTON. This Journal **100:** 685, 1932.
Ibid. **104:** 399, 1933.
SWINGLE, W. W. ET AL. This Journal **108:** 159, 1934.
ZWEMER, R. L. Endocrinol. **18:** 161, 1934.
ZWEMER, R. L. AND R. C. SULLIVAN. Endocrinol. **18:** 97, 1934.

THE EFFECT OF URINE FROM PREGNANT WOMEN ON THE OVARY-STIMULATING POTENCY OF THE HYPOPHYSES OF RABBITS AND RATS

LE ROY GOODMAN

From the Department of Obstetrics, Harvard Medical School, Boston Lying-in Hospital, Boston, Mass.

Received for publication August 13, 1934

Evans, Meyer and Simpson in 1932 reported a striking difference between the quantitative effect of hypophyseal extract and of urine-concentrate from pregnant women on the ovaries of immature rats. Hypophyseal extract caused an increase in ovarian weight in proportion to the amount injected, while urine-concentrate was followed by an initial augmentation in weight which remained at a lower level without any subsequent gain, in spite of the administration of further quantities. When both were administered simultaneously, however, the weights of the ovaries became greater than could be accounted for merely by an additive effect. In the latter case, according to their reasoning, the hypophyseal hormone which was considered to be mainly a growth hormone was transformed into a sex hormone. Leonard, in the same year, published data to show that his hypophyseal sex hormone fraction could be similarly activated by pregnancy urine, so that the effect of both given together was greater than that due to either one given alone in equivalent quantities. Collip, Selye and Thomson (1933) suggested from their experiments that the hypophysis, as well as pregnancy urine, contains a substance "complementary" to the sex-stimulating hormones, derived from the hypophysis and pregnancy urine.

Since the procedure in preparing hypophyseal extracts outside the body might conceivably modify their sex potency or render them susceptible to the activating influence of pregnancy urine, the following experiments were designed to determine the effect, if any, of pregnancy urine on the hypophysis in the live animal. Briefly, pregnancy urine was injected into a series of rabbits for varying periods, at the end of which time the hypophyses of these rabbits were implanted daily into immature female rats and assayed by their effects on the weights of the ovaries.

PROCEDURE. The experimental procedure consisted in giving rabbits of approximately the same ages and weights 10 cc. of pregnancy urine intravenously daily for three or for thirty consecutive days. The urine

used was shaken for five minutes with three parts of ether to remove toxic products and the major portion of the estrin. Virgin rabbits which had been isolated were selected in order to insure an initial constancy in the sex potency of their hypophyses, derived from the fact that the rabbits had previously been neither pregnant nor pseudo-pregnant. Moreover, the series of rabbits consisted of Blue Bevern stock of approximately equal age and weight kept under uniform conditions of housing and feeding. Several series of spayed male and female rabbits were used. At the end of the injection period the animals were killed by bleeding to death; the hypophyses were removed aseptically and split into halves which were immediately implanted into each of two litter-mate, 21-day old female rats. For controls, halves of hypophyses of untreated rabbits were implanted into two other rats of the same litter at the same time. Implantations were made for five to ten consecutive days following the method of Smith (1926). At the end of the implantation period all of the litter-mate rats were killed on the same day, the ovaries carefully dissected free and weighed on a chemical balance.

For comparison with the rabbit series, a series of adult rats of both sexes, gonadectomized and normal, was similarly given three daily subcutaneous injections of one cubic centimeter of ether-extracted urine from pregnant women. The hypophyses of this series of rats were assayed in the same manner as the rabbits' hypophyses by implanting the whole glands into immature rats for ten consecutive days.

It should be added that the urine from pregnant women used in these experiments was on each occasion a fresh pooled quantity collected during a single day from a subject in the first third of pregnancy. The urine was kept in the refrigerator. The 10 cubic centimeter samples injected after extraction with ether gave no oestrous response in vaginal smears of spayed female rats.

Experiment I. The effect of urine from pregnant women on the potency of the hypophyses of normal female rabbits. One series of ten, and three series of six female rabbits were given daily intravenous injections of pregnancy urine for three days. A further series of six were given daily injections for thirty days. In all there were thirty-four treated donors and a similar number of controls in this experiment. The hypophyses were assayed on twenty rats, ten for the treated rabbits' hypophyses and ten for the untreated hypophyses. The results of the assay, expressed in weights of the rats' ovaries, are presented in table 1. It is apparent that the hypophyses of treated rabbits induced greater weight changes in rat ovaries than were produced by the normal hypophyses. The increase in percentages resulting from implantation of treated hypophyses ranged from 42 to 250 per cent with an average increase of 113.5 per cent. This augmentation of a sex potency was present in the hypophyses of rabbits

TABLE 1

Assay of ovary-stimulating factors of hypophyses of normal female rabbits with and without previous intravenous injection of urine from pregnant women

RAT LITTER NUMBER	TREATMENT OF DONOR RABBITS	NUMBER OF RAT	NUMBER OF DAYS OF IMPLANTATION OF HYPOPHYSES	COMBINED WEIGHTS OF OVARIES OF RATS RECEIVING HYPOPHYSES OF TREATED RABBITS	COMBINED WEIGHTS OF OVARIES OF RATS RECEIVING HYPOPHYSES OF UNTREATED RABBITS
				mgm.	*mgm.*
I	20 rabbits				
	Urine for 3 days	Rat 1	10	55.0	
	Urine for 3 days	Rat 2	10	53.5	
	Untreated controls	Rat 3	10		40.0
	Untreated controls	Rat 4	10		36.0
	Average weight of both ovaries			54.25	38.0
	Percentage of difference			42.0%	
II	12 rabbits				
	Urine for 3 days	Rat 5	6	16.25	
	Urine for 3 days	Rat 6	6	20.2	
	Untreated controls	Rat 7	6		6.6
	Untreated controls	Rat 8	6		5.5
	Average weight of both ovaries			18.3	6.0
	Percentage of difference			250%	
III	12 rabbits				
	Urine for 3 days	Rat 9	6	14.5	
	Urine for 3 days	Rat 10	6	15.1	
	Untreated controls	Rat 11	6		5.0
	Untreated controls	Rat 12	6		7.0
	Average weight of both ovaries			14.7	6.0
	Percentage of difference			145%	
IV	12 rabbits				
	Urine for 3 days	Rat 13	6	25.0	
	Urine for 3 days	Rat 14	6	14.0	
	Untreated controls	Rat 15	6		12.1
	Untreated controls	Rat 16	6		12.0
	Average weight of both ovaries			19.5	12.0
	Percentage of difference			62.5%	
V	12 rabbits				
	Urine for 30 days	Rat 17	6	7.0	
	Urine for 30 days	Rat 18	6	7.8	
	Untreated controls	Rat 19	6		4.2
	Untreated controls	Rat 20	6		5.6
	Average weight of both ovaries			7.5	4.4
	Percentage of difference			68.4%	

Average of all percentage differences	113.5%
Total number of treated donor rabbits	34
Total number of control donor rabbits	34
Total number of recipient rats	20

treated for three as well as for thirty days. The highest, as well as the lowest percentages of increase, occurred in the series treated for three days.

Experiment II. The effect of urine from pregnant women on the potency of the hypophyses of spayed female rabbits. a. In a further experiment five female rabbits, ovariectomized for one month, were given three daily

TABLE 2

Assay of ovary-stimulating factors of hypophyses of ovariectomized rabbits with and without previous intravenous injection of urine from pregnant women

10 rabbits

TREATMENT OF DONOR RABBITS	NUMBER OF DAYS OF IMPLANTATION OF HYPOPHYSES	NUMBER OF RAT	COMBINED WEIGHTS OF OVARIES OF RATS RECEIVING HYPOPHYSES OF TREATED RABBITS	COMBINED WEIGHTS OF OVARIES OF RATS RECEIVING HYPOPHYSES OF UNTREATED RABBITS
			mgm.	*mgm.*
Urine for 3 days	5	Rat 1	48.4	
Urine for 3 days	5	Rat 2	32.0	
Untreated controls	5	Rat 3		14.5
Untreated controls	5	Rat 4		14.7
Average weight of both ovaries			40.2	14.6
Percentage of difference			175.3%	
Total number of treated donor rabbits				5
Total number of control donor rabbits				5
Total number of recipient rats				4

Assay of ovary-stimulating factors of hypophyses of ovariectomized rabbits as compared with normal rabbits

10 rabbits

Ovariectomized (1 mo.)	5	Rat 1	18.5	
Ovariectomized (1 mo.)	5	Rat 2	17.0	
Non-spayed controls	5	Rat 3		7.3
Non-spayed controls	5	Rat 4		10.0
Average weight of both ovaries			17.75	8.65
Percentage of difference			105.1%	
Total number of treated spayed rabbits				5
Total number of control spayed rabbits				5
Total number of recipient rats				4

intravenous injections of urine from pregnant women and their hypophyses assayed as above. A similar number of spayed, untreated control rabbits were also tested on litter-mate rats. The results of this experiment are shown in table 2. The average ovarian weight of the treated group was 175.3 per cent greater than in the untreated group.

b. A similar series of five spayed, but untreated female rabbits, whose hypophyses were assayed on rats, together with a comparable number of non-spayed controls, is presented in the lower half of table 2. The hypophyses of these spayed but untreated rabbits show an increase of 105.1 per cent in their power to stimulate rat ovaries. A similar increase has recently been found for rabbits spayed for thirty-three days by Smith, Severinghaus and Leonard (1933), using a different method of assay than ours, and is in agreement with the results of Engle (1929) and Evans and Simpson (1929), using spayed rats, instead of rabbits.

It will be seen from comparing procedure *a* with *b* that the sole difference in the two sets of experiments is that in *a* the rabbits received injections of

TABLE 3

Assay of ovary-stimulating factors of hypophyses of male rabbits with and without previous intravenous injection of urine from pregnant women

10 rabbits

TREATMENT OF DONOR RABBITS	NUMBER OF DAYS OF IMPLANTATION OF HYPOPHYSES	NUMBER OF RAT	COMBINED WEIGHTS OF OVARIES OF RATS RECEIVING HYPOPHYSES OF TREATED RABBITS	COMBINED WEIGHTS OF OVARIES OF RATS RECEIVING HYPOPHYSES OF UNTREATED RABBITS
			mgm.	*mgm.*
Urine for 3 days	5	Rat 1	16.0	
Urine for 3 days	5	Rat 2	18.0	
Untreated controls	5	Rat 3		11.0
Untreated controls	5	Rat 4		10.0
Average weight of both ovaries			17.0	10.5
Percentage of weight increase			60.1%	
Total number of treated male rabbits				5
Total number of control male rabbits				5
Total number of recipient rats				4

urine. Further it will be noted that the hypophyses of series *a* caused a greater weight change in the rats' ovaries than in series *b*.

Experiment III. The effect of urine from pregnant women on the potency of the hypophyses of normal male rabbits. Five male rabbits were given three daily intravenous injections of pregnancy urine and their hypophyses were compared in a similar manner to the previous experiments with those of a like number of controls. The results are shown in table 3. The ovarian weights of the rats receiving hypophyses of treated male rabbits showed a 60.1 per cent increase over the controls. The number of rats used for assay in this series is too small, however, to obviate experimental error. The data suggest, nevertheless, that the percentage of increase of ovarian weight induced by stimulated male hypophyses is less than the increase produced by treated female hypophyses (table 1).

Experiment IV. The effect of urine from pregnant women on the potency of the hypophyses of normal male and female rats. Finally the experiments recorded in table 4 show the effects of hypophyses of donor rats upon recipient rats, the difference from the previous series being that rats are

TABLE 4

Assay of ovary-stimulating factor of hypophyses of normal female and male rats with and without previous subcutaneous injection of urine from pregnant women

	TREATMENT OF DONOR RATS	NUMBER OF RAT RECIPIENT (SAME LITTER)	NUMBER OF DAYS OF IMPLANTATION OF HYPOPHYSES	WEIGHTS OF OVARIES OF RATS RECEIVING HYPOPHYSES OF TREATED RATS	WEIGHTS OF OVARIES OF RATS RECEIVING HYPOPHYSES OF UNTREATED RATS
				mgm.	*mgm.*
A	20 rats				
	Treated normal females—5 days	Rat 1	10	66.0	
	Untreated normal females	Rat 2	10		73.0
	Percentage of decrease			−10%	
B	20 rats				
	Treated spayed females—5 days	Rat 3	10	185.0	
	Untreated spayed females	Rat 4	10		121.0
	Percentage of increase			+53%	
C	20 rats				
	Treated normal males—5 days	Rat 5	10	46.0	
	Untreated normal males	Rat 6	10		146.0
	Percentage of decrease			−31.7%	
D	20 rats				
	Treated castrated males—5 days	Rat 7	10	155.0	
	Untreated castrated males	Rat 8	10		153.0
	Percentage of increase			+1.3%	

Percentage of increase of normal males over normal females	100.0%
Percentage of increase of castrated over non-castrated males	4.7%
Percentage of increase of spayed over non-spayed females	65.8%
Total number of treated donor rats	40
Total number of control donor rats	40
Total number of test rats	8

substituted for rabbits. Here both male and female donors were treated daily by subcutaneous instead of intravenous injections of one cubic centimeter of pregnancy urine for five consecutive days. As in the previous experiments, both normal and gonadectomized donor animals were used. There were, in all, forty treated donors and an identical number of controls.

Hypophyses of ten treated rats were used in each of four experiments, viz., hypophyses of *a*, treated normal females; *b*, treated spayed females; *c*, treated normal males, and *d*, treated castrated males. The urine-treated rats as well as the controls were approximately 150 days old, the test rats were 21 days old as in the previous experiments. These series were compared for control with hypophyses of *a*, untreated normal females; *b*, untreated spayed females; *c*, untreated normal males, and *d*, untreated castrated males. The gonadectomized rats had been operated upon three weeks previously. The eight recipient test rats were of the same litter. It can be seen from examination of table 4 that, in contrast to rabbits, the treatment with pregnancy urine decreased instead of increasing the potency of normal adult male and female rats' hypophyses. It diminished the potency of normal female rats' hypophyses only 10 per cent (expt. a) whereas it decreased that of normal male rats 22 per cent (expt. c). On the other hand, treatment with pregnancy urine increased by 53 per cent the potency of the hypophyses of spayed female rats (expt. b), whereas in the similar spayed, treated males (expt. d), there was no significant change in potency. The results in normal females are in agreement with those of Kuschinsky (1931), who used a different method of assay. Except for the increase in the spayed females, these results are also in agreement with those of Leonard (1933), who investigated the effect of injection of prolan on the hypophyses of normal and gonadectomized rats. He found decreases in the normal and no significant change in either gonadectomized group.

In addition the data show that normal male rats' hypophyses are 100 per cent more potent than female hypophyses. Contrariwise, the hypophyses of untreated gonadectomized males caused only a 4.7 per cent increase in ovarian weight, while the hypophyses of untreated gonadectomized female controls led to a 65.8 per cent increase in ovarian weight. These results agree in the main with those of other workers (Engle, 1929; Evans and Simpson, 1929).

Discussion. From the data recorded in tables 1 and 3 it follows that an increase in ovary-stimulating potency occurs in the hypophyses of normal adult male and female rabbits following the intravenous injection of ether-extracted human urine of pregnancy. These findings in rabbits are in marked contrast to the results obtained in normal male and female rats. In the latter, the sex-stimulating potency is decreased, instead of being increased, following injection of pregnancy urine, according to the account of Leonard (1933), as well as from the results of the present series of rats. Our findings and Leonard's upon the rat are in good accord, although he used the single implant method in mice as a method of assaying the hypophyses, whereas we used repeated daily implants into other rats as our method of assay. Leonard interpreted the decreased size of the ovaries, resulting from the implantation of hypophyses of rats

treated with prolan, to the effect that the prolan given stimulated the ovaries or testes of the donor rats to excess production of oestrin or male sex hormone as the case might be. The oestrin and male sex hormone in turn, according to his further reasoning, then acted upon the hypophysis to depress it; consequently, when the hypophyses were implanted into a test-mouse, the ovaries did not respond by an increase in weight to the same extent as did those given hypophyses of untreated controls. Obviously in the present series of rabbits in which the ovaries of the test rats increased in weight more than did those of the controls, the same interpretation is not applicable. There is, apparently, a difference in the factors operating in the rabbit and rat. It seems unlikely that the differences in the methods used are responsible for the results, for in our series of rabbits and rats, at least, the same method was employed. Species differences in the reactions of the hypophyses and ovaries of rabbits and rats are more likely responsible. Rabbits' ovaries do react differently from rats' ovaries to stimulation by pregnancy urine. The ovaries of our donor rabbits, after three days of injection of oestrin-free pregnancy urine, showed excessive stimulation, evidenced by the formation of numerous recent corpora lutea and corpora hemorrhagica. The donor rats' ovaries, on the other hand, reacted by a proliferation of follicles and a certain degree of luteinization. In agreement with Leonard it may be considered that the major response of the rats' ovaries under the circumstances is the production of oestrin which inhibits sex hormone production by the hypophysis. The marked formation of corpora lutea in rabbits' ovaries, on the contrary, indicates a different response. The rabbit's hypophysis, moreover, in contrast to the rat's, responds to the changes in the ovaries by an increase in potency, indicating that the ovarian reaction is not primarily one by which oestrin is released. In fact, the hypophyseal response resembles the effects obtained after simple gonadectomy, and suggests that the effect of urine on rabbits' ovaries may be to produce a condition of virtual castration as far as the hypophysis is concerned. That gonadectomy by itself increases hypophyseal potency in rabbits has been shown by Smith, Severinghaus and Leonard (1933) as well as by the present corroborative experiments.

Our experience with rabbits indicates, moreover, that, besides influencing the hypophysis through the ovarian response, the active principle of ether-extracted pregnancy urine exercises a direct action on the hypophysis as well. This is suggested by the observation that the ovary-stimulating potency of the hypophysis is greater in spayed female rabbits which have received urine. This result suggests strongly an action of pregnancy urine directly upon the rabbits' hypophysis without the intermediation of the gonad. This evidence is in keeping with the conceptions of Evans, Meyer, and Simpson (1932) and of Collip, Selye, and Thomson (1933) referred to in the introduction, regarding a complementary or synergistic

relationship between prolan and the sex hormone of the anterior lobe. No similar direct effect upon the hypophysis has been demonstrated for rats, unless the present observation, that the potency of the hypophyses of spayed female rats receiving prolan is greater than in untreated spayed females, is borne out by further experiments. It should be recalled that our spayed female rats exhibited increased hypophyseal potency following injection of pregnancy urine in contrast to the negative results of Leonard's spayed rats so that further series of treated spayed females' hypophyses should be tested to settle this point definitely.

SUMMARY

1. In rabbits, the intravenous injection of ether-extracted human urine of pregnancy increases the ovary-stimulating potency of the hypophyses of normal adult male and female rabbits and of spayed adult female rabbits.

2. Contrariwise, in rats the subcutaneous administration of ether-extracted human urine of pregnancy decreases the ovary-stimulating potency of the hypophyses of adult male and female rats and does not change the potency of the hypophyses of castrated adult male rats.

3. In a small series of spayed female rats the subcutaneous administration of ether-extracted human urine of pregnancy increases the ovary-stimulating potency of the hypophyses.

4. Spaying, without treatment with urine, increases the ovary-stimulating potency of the hypophyses of both rabbits and rats.

5. The ovary-stimulating potency of hypophyses of normal adult male rats is greater than that of adult females. After gonadectomy the increase in potency is greater in the females.

6. The present experiments suggest that in the rabbit the action of human urine of pregnancy upon the hypophysis is partly indirect through the gonads and partly direct upon the hypophysis itself. The influence upon the hypophysis is similar to the effect of gonadectomy.

7. In the rat on the contrary the effect of pregnancy urine upon the hypophysis appears to be an indirect one only through the gonad. This gonadal influence differs from that occurring in the rabbit in that it is presumably due to the inhibitory action of oestrin or male sex-hormone.

REFERENCES

COLLIP, J. B., H. SELYE AND D. L. THOMPSON. Virchow's Arch. **290:** 23, 1933.
ENGLE, E. T. This Journal **88:** 101, 1929.
EVANS, H. M. AND M. E. SIMPSON. This Journal **89:** 371, 1929.
This Journal **89:** 375, 1929.
EVANS, H. M., K. MEYER AND M. E. SIMPSON. This Journal **100:** 141, 1932.
KUSCHINSKY, G. Arch. f. Exp. Path. u. Pharmacol. **162:** 183, 1931.
LEONARD, S. L. Proc. Soc. Exp. Biol. **30:** 403, 1932.
Anat. Rec. **57:** 45, 1933.
SMITH, P. E. Proc. Soc. Exp. Biol. **24:** 1931, 1926.
SMITH, P. E., A. E. SEVERINGHAUS AND S. L. LEONARD. Anat. Rec. **57:** 177, 1933.

THE SURVIVAL OF SALT-TREATED ADRENALECTOMIZED RATS

ROBERT GAUNT, CHARLES E. TOBIN AND JO HOWLAND GAUNT

From the Department of Biology, College of Charleston, S. C., and The Biological Laboratory, Cold Spring Harbor, N. Y.

Received for publication October 23, 1934

The fact, first clearly established by Stewart and Rogoff (1925), that the administration of large quantities of salt solution to adrenalectomized animals delayed the appearance of adrenal insufficiency symptoms, has been confirmed by several groups of workers (Banting and Gairns, 1926; Corey, 1927; Marine and Baumann, 1927; Swingle, Pfiffner, Vars and Parkins, 1934; and Zwemer, 1934). Such treatment in the dog and cat, however, is merely palliative and does not indefinitely substitute for the adrenal cortical hormone. Recent work done by Loeb (1933) and Harrop et al. (1933a, b) indicates that benefit is obtained not only in animals but also in cases of Addison's disease treated with salt or salt solutions.

Rubin and Krick (1933) reported experiments performed on adrenalectomized rats which indicated that in this form salt treatment is much more effective than others had found it to be for the cat and dog. By allowing their animals to drink only a solution of 0.0329 per cent $CaCl_2$, 0.015 per cent MgCl, 0.7 per cent NaCl, and 0.035 per cent KCl, death from adrenalectomy was prevented in all of their eight treated cases. These results, obtained on animals whose normal life-span after adrenalectomy, was, according to the authors, not longer than 10 days, would indicate that in the rat a salt solution would substitute completely for the hormone of the adrenal cortex.

The present experiments were designed to study further the effectiveness of various forms and amounts of salt treatment in preventing the symptoms of adrenal insufficiency in rats of different ages.

METHODS. In all of our animals, with the exception of those given saline injections, either the Rubin-Krick salt solution, mentioned above (which will subsequently be referred to as the "R-K solution"), or 0.9 per cent NaCl solution was given the animals to drink. No pure water was permitted. The experimental animals were fed either our regular diet or this diet with salt added. The regular diet is made as follows: 9 parts by weight of GLF Calf Meal, and 1 part ground meat and bone scrap; plus small quantities of cod liver oil, yeast and lettuce. One per cent NaCl is added by the manufacturers to the Calf Meal, making the total NaCl added to

the mixture 0.9 per cent. The total NaCl content of the diet, considering the salt in its constituents, is 2.53 per cent.[1]

In part of the cases, a diet designated as the "salt diet," was prepared by adding to the regular diet 1.6 per cent NaCl.

Five types of treatment were tried, the treatment beginning, except in cases specifically mentioned, at the time of operation: 1, regular diet and normal saline solution to drink; 2, salt diet and normal saline solution to drink; 3, regular diet and R-K solution to drink; 4, salt diet and R-K solution to drink; 5, regular diet and distilled water to drink, plus intraperitoneal injections of normal saline (10 cc./day).

Daily weight records were kept of all animals, and in most cases records of the daily food and fluid consumption. A series of 23 adult animals were treated by certain of these means, and the preliminary results published (Gaunt, Tobin and Gaunt, 1934). The normal life-span of adults varies widely, however, and anything that will extend life will oftentimes permit the hypertrophy of accessory adrenals, resulting in indefin te survival (Gaunt and Gaunt, 1934). We, therefore, turned to the use of animals weaned at 28 days of age and adrenalectomized at 30 days of age, for the main part of this work. The young animals are more susceptible to adrenalectomy than the mature ones, and the length of life after adrenalectomy is less variable. The effects of beneficial treatment are, for this reason, more clearly and immediately apparent. Forty-one 30-day-old animals, averaging 57.6 grams weight, were adrenalectomized and given salt treatment. Forty-four controls, littermates of the treated animals, were adrenalectomized and given no treatment.

Effect of treatment on survival—30-day-old animals. The five types of treatment tried were all undoubtedly effective in extending the lives of part of the animals, and are therefore plotted together in figure 2, which is to be compared with the graph of the untreated, littermate, controls in figure 1.

In the controls 89 per cent were dead within 12 days after operation, 95.6 per cent were dead by the 34th day and two (4.4 per cent) survived indefinitely and showed accessories at autopsy. The average survival of those that died was 8 days (fig. 1).

In the salt-treated animals, less than half, 43.9 per cent, died within 3 to 14 days, the average survival being 9.0 days. No deaths occurred after this during treatment. Those that did succumb while on the salt diets apparently got little if any benefit from the treatment, because the average survival and distribution of deaths was very similar to that of the controls.

In striking contrast to the situation in the controls, however, was the

[1] The NaCl analysis was made by Dr. B. F. Robertson of the Clemson Agricultural College, Clemson, S. C.

fact that 56.1 per cent of the salt-treated animals were alive at the end of the 30-day period of treatment, at which time only 6.5 per cent of the controls were alive. Treatment was discontinued in all cases on the thirtieth day, and immediately thereafter deaths began to occur. Forty-eight and eight-tenths per cent of the total number of animals died within 18 days after the salt solutions were withdrawn. Seven and three-tenths per cent (3 animals) continued to grow after treatment was stopped and lived in good condition until killed for autopsy 2 months later, at which time accessories were found (fig. 2).

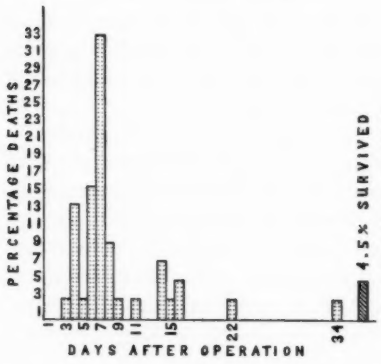


Fig. 1

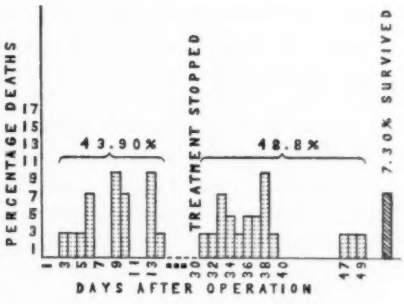


Fig. 2

Fig. 1. Shows the life-span of 44 control rats, adrenalectomized at 30 days of age. The dotted columns represent the percentage of animals dying on given days. The cross-lined column represents the percentage which survived indefinitely.

Fig. 2. Shows the life-span of 41 rats, adrenalectomized at 30 days of age, and treated for 30 days after operation with various salt solutions. All of the deaths observed during treatment occurred by the fourteenth day. At that point the abscissa of the graph is broken and goes to 30 days, at which time treatment was discontinued and many deaths began to occur. The dotted columns represent the percentage of animals dying on any given day. The cross-lined column represents the percentage which survived indefinitely.

Effectiveness of different types of treatment. A comparison of the effects of the five different variations of the treatment indicates that there was no benefit from the "salt diet" (to which 1.6 per cent NaCl had been added) as compared to the regular diet. Either normal saline or the R-K solution with regular diet was just as effective as with the salt diet.

The R-K solution, however, was probably more effective than normal saline—which was also the tentative conclusion of Rubin and Krick. Seven (41.3 per cent) of the 17 animals given normal saline to drink survived during the course of treatment, and the average life of those that

died was 8.1 days; while nine (70.3 per cent) of the 13 animals given the R-K mixture survived a similar period of time. Those dying showed an average life-span of 10.2 days.

Intraperitoneal injections of normal saline, if properly administered, are perhaps slightly more effective than giving this solution to drink, although the total NaCl consumed was considerably greater when given orally. Sixty-three and seven-tenths per cent of the 11 saline-injected animals survived during the 30 days of their treatment, compared to 41.3 per cent of the 17 given normal saline to drink. In the saline-injected animals the dosage was 10 cc. of 0.9 per cent NaCl in divided doses twice daily. To prevent a fatal diarrhea it was found necessary to begin with an injection of 1 cc. per day four or five days before operation, and each day increase the dosage by 1 cc. until the desired maximum was reached. No indication of diarrhea or other ill effects followed such treatment.

Maintenance of growth rate and other effects of treatment. It is obvious that in the majority of cases salt-treatment markedly prolonged the lives of adrenalectomized rats, but observation of the activity of the animals, and their weight records, indicate that normal health and growth was not maintained. Many of those kept alive with salt, compared to normal rats of same age, were somewhat listless and inactive. The average weight of the animals used at 30 days of age, the time of operation, was 57.6 grams. At 60 days of age, the time treatment was discontinued, the average weight of those surviving that long was 119.3 grams, an increase of 107 per cent. In our normal rats there is an increase in weight of 180 per cent or more during the second 30 days of life. Clearly the salt treatment did not maintain the normal growth rate.

In animals dying during the course of salt treatment, particularly those receiving salt injections, diarrhea occurred during the terminal stages of their insufficiency more frequently than it does in untreated, operated animals. Diarrhea was not seen before those terminal stages. In some cases, apparently, the condition of adrenal insufficiency does not allow the animals to utilize the needed salt solutions. This may be the reason for our inability, to be discussed later, to revive animals in the terminal stages of adrenal insufficiency by salt treatment.

Food and fluid consumption. On the average the animals on salt solutions drank and ate considerably more than the control adrenalectomized animals on distilled water (table 1). Adrenal insufficiency, however, was developing in the controls in a much higher percentage of cases than in the treated animals; and, as is well known, the fluid and food consumption of animals coming down with adrenal insufficiency is reduced. Therefore, whether the lesser food and fluid consumption in the controls is the cause of, or was caused by, the greater incidence of adrenal insufficiency is not clear. In the observation of individual cases, however, it is obvious that

those animals withstood adrenalectomy best which drank the most solution. Normal animals given normal saline to drink consume about one-fourth more than those given distilled water.

When the animals drinking salt solutions were returned to normal diet the fluid consumption dropped, on the average, by about half. Again this drop was associated with the development of fatal adrenal insufficiency in the majority of cases. In those cases surviving indefinitely there was, surprisingly, sometimes little or no change in fluid consumption when returned to distilled water.

TABLE 1

Average daily food and fluid consumption of salt-treated and untreated 30-day-old adrenalectomized rats

	FIRST 10 DAYS		SECOND 10 DAYS		THIRD 10 DAYS		Treatment discontinued	FOURTH 10 DAYS	
	Fluid cc./day	Food gm./day	Fluid cc./day	Food gm./day	Fluid cc./day	Food gm./day		Fluid cc./day	Food gm./day
Untreated controls	12.7 (7 animals)	5.9	19.0 (3 animals)	9.9	25.0 (2 animals)	12.0			
Salt-treated	16.0 (17 animals)	8.7	25.9 (10 animals)	11.1	31.6 (10 animals)	14.4		17.3 (10 animals)	10.4
Saline-injected	9.0 (8 animals)		13.0 (5 animals)		15.0 (5 animals)			20.9 (5 animals)	

The above table shows the average food and water consumed by the 30-day-old animals during the four 10-day periods after adrenalectomy. The numbers of animals involved in the different time periods vary, especially because at the end of the first 10 days many animals were dead (see figs. 1 and 2). The record of no animal is included during the last 48 hours of its life, at which time consumption figures drop precipitously.

The controls and salt-injected animals received distilled water to drink, but the latter received 10 cc./day injections of normal saline. The "Salt-treated" animals received either the Rubin-Krick solution or normal saline to drink.

The food records are fairly accurate comparatively, but no correction was made for a small amount of inevitable waste in the use of finely-ground food.

The series of animals given normal saline injections of 10 cc. per day and distilled water to drink, drank less, by about half, than those drinking salt solutions (table 1).

Salt-treatment in mature adrenalectomized rats. The types of salt treatment used on the 30-day animals were, with the exception of intraperitoneal saline injections, tried also on a series of 23 adult, adrenalectomized rats, weighing from 170 to 211 grams. It was not apparent in these cases that one treatment was more effective than another. These salt-treated mature animals were compared to a series of 24 untreated, mature, adrenalec-

tomized controls, and with the immature, salt-treated animals previously discussed. Comparative data are summarized in table 2.

The salt treatment was more effective in the adults than in the young animals, both in keeping the animals alive during the treatment, and in permitting indefinite survival after treatment. Five (21.75 per cent) of the adult, salt-treated animals died during the course of treatment, compared to 43.9 per cent of the 30-day animals. Twelve (52.2 per cent) of the mature animals survived indefinitely, compared to 7.32 per cent of the young rats. An almost identical result was obtained after we treated adult animals of this colony with the cortical hormone. Approximately half of them, by the development of accessories, lived indefinitely after hormone treatment was stopped, whereas in untreated adults 12.5 per cent indefinite survival is the highest we have ever obtained in any control series. In the adults of this colony it seems that any remedial agent

TABLE 2

Survival of adult and 30-day-old, salt-treated, adrenalectomized rats; and adult, untreated, adrenalectomized controls

	TOTAL NO. OF CASES	NO. DEATHS IN FIRST 30 DAYS	AV. SURVIVAL OF RATS THAT DIED ON TREATMENT IN DAYS, WITH STANDARD ERRORS	Treatment discontinued at 30 days	NO. DEATHS AFTER 30 DAYS	NO. SURVIVING INDEFINITELY
Salt-treated adults.....	23	5 (21.7%)	20.6 ±1.8		6 (26.1%)	12 (52.2%)
Salt-treated 30-day-old...	41	18 (43.9%)	9.0 ±0.77		20 (48.8%)	3 (7.3%)
Untreated adult controls.	24	18 (75.0%)	17.8 ±2.5		3 (12.5%)	3 (12.5%)

that will extend life appreciably after adrenalectomy, will, probably by allowing time for the hypertrophy of accessories, permit the indefinite survival of about half of the cases. Why this is not likewise true in immature animals is not clear. If the accessories are present during all ages, as a result of the diffuse embryological origin of the adrenal cortex, then it would seem that they should be as capable of hypertrophy in a young animal as in an adult. It may, however, be true that the demands of a growing animal upon its cortical tissue are greater than in an adult, and that the rate of hypertrophy cannot meet the needs of the animal.[2]

Revival of animals with symptoms of adrenal insufficiency with salt. Rubin and Krick did not begin their treatment with salt-solutions until their rats

[2] Kutz et al. have recently reported, in connection with a study of the oestrus cycle in adrenalectomized rats given the Rubin-Krick solution, that the treatment kept only 18 of 31 animals alive.

were in "marked insufficiency." They reported that, "even if this fluid was not given until within 2 to 3 hours before the time that death would have ordinarily occurred, the animals could be restored to a normal state within 4 to 5 hours after its administration." In part of their cases the animals were so weak that the solution was given orally by pipette. This is a striking result, because from two to three hours before death a mature adrenalectomized rat is almost always prostrate, having recurrent convulsions, and has lost from 4° to 12°F. of its body temperature. In such stages it is not always possible to revive them with cortical extract.

We attempted to revive 14 animals, the majority of which were adults, with salt treatment. Most of them were in the terminal stages of adrenal insufficiency, i.e., prostration was present or beginning and the body temperature was falling, but not lower than 92°F. It is not possible to predict with any accuracy how long these animals would have lived if untreated, but probably from 2 to 12 hours. The method of feeding salt solutions (either normal saline or the R-K mixture) by mouth with pipette was tried, sometimes by itself, and in other cases combined with intraperitoneal injections of normal saline (3 to 8 cc.). In 12 of the 14 cases death was apparently not delayed, and no certain remedial effects of the treatment were observed. In two cases revival was effected, and was followed by death three days later, as, once revived, no further treatment was given.

It is probable that resuscitation could be obtained in a higher percentage of cases if treatment were begun when the symptoms of adrenal insufficiency were less severe.

Discussion. Our results confirm those of Rubin and Krick in the general conclusion that salt administration will assist adrenalectomized rats in maintaining life. We were, however, unable to duplicate their result of keeping 100 per cent of the animals alive for as long as treatment was continued, nor were we as successful in the revival of animals with terminal symptoms.

The general finding for the adrenalectomized cat and dog is that salt treatment will prolong but not indefinitely maintain life. The rat does not exactly conform to this rule, either according to our results or those of Rubin and Krick. In our findings 43.9 per cent of the 30-day-old animals were not appreciably benefited by the salt treatment, and died during the first 14 days. On the other hand, 48.8 per cent were obviously greatly assisted, lived until treatment was stopped, and died thereafter. In most of these cases it is probable life could have been extended further if treatment had been continued.

Why some animals are helped and others are not remains a problem. In part at least of these cases there may have been present minute cortical accessories, insufficient in size to maintain life unassisted. Such acces-

sories are present in adults of this colony in at least half of the cases, as is apparent from the number surviving indefinitely after cortical hormone or salt treatment. Thus, it might be true that part of those animals surviving during salt administration and dying after it was discontinued, did so by virtue of the fact that they were receiving minute amounts of the cortical hormone from accessories, along with the salt. This idea is in conformity with the recent human studies of Addison's disease, where it is found that in some cases salt will either restore and keep a patient in good condition or greatly reduce the amount of cortical hormone needed (Loeb, Harrop et al.). In Addison's disease there is generally at least some cortical tissue present (Rowntree and Snell, 1931).

SUMMARY

1. Salt solutions, either normal saline or a mixture of salts recommended by Rubin and Krick, given to adrenalectomized rats orally, are of help in extending the life-span. Daily intraperitoneal injections of normal saline solution are also beneficial. Treatment was begun at the time of operation.

2. The survival of a series of 41 salt-treated rats adrenalectomized at 30 days of age was studied and compared with a series of 44 untreated controls. A series of 23 salt-treated, adrenalectomized adults was compared with 24 untreated, adult controls. In all cases treatment was continued for 30 days after adrenalectomy, if the animals lived that long.

3. In the 30-day-old animals, 43.9 per cent of the salt-treated animals died during the 30-day period of treatment, all deaths occurring by the 14th day. Forty-eight and eight-tenths per cent died after treatment was stopped, and 7.3 per cent lived indefinitely.

4. In the litter-mate untreated controls, 93.5 per cent died during the first 30 days, and 4.34 per cent lived indefinitely.

5. In the salt-treated adults 21.7 per cent died during the 30-day treatment period, 26.1 per cent died after treatment was stopped, and 52.2 per cent lived indefinitely.

6. In the untreated adult controls, 75.0 per cent died during the first 30 days after operation, and 12.5 per cent lived indefinitely.

7. The Rubin-Krick solution is probably more effective than normal saline solution in extending the life-span.

8. Animals kept alive by salt-treatment grow at a definitely sub-normal rate.

9. It was rarely possible to resuscitate rats in the terminal stages of adrenal insufficiency with salt solutions.

The assistance and advice given by Dr. W. W. Swingle and Mr. William Parkins of Princeton University in carrying out this investigation has been greatly appreciated.

REFERENCES

BANTING, F. G. AND S. GAIRNS. This Journal **77**: 100, 1926.

COREY, E. L. This Journal **79**: 633, 1927.

GAUNT, R. This Journal **103**: 494, 1933.

GAUNT, R. AND J. H. GAUNT. Proc. Soc. Exp. Biol. and Med. **31**: 490, 1934.

GAUNT, R., C. E. TOBIN AND J. H. GAUNT. Proc. Soc. Exp. Biol. and Med. **32**: 134, 1934.

HARROP, G. A., L. J. SOFFER, R. ELLSWORTH AND J. H. TRESCHER. J. Exp. Med. **58**: 17, 1933a.

HARROP, G. A., A. WEINSTEIN, L. J. SOFFER AND J. H. TRESCHER. J. A. M. A. **100**: 1850, 1933b.

KUTZ, R. L., T. MCKEOWN, AND H. SELYE. Proc. Soc. Exp. Biol. and Med. **32**: 331, 1934.

LOEB, R. F. Proc. Soc. Exp. Biol. and Med. **30**: 808, 1933.

MARINE, D. AND E. J. BAUMANN. This Journal **81**: 86, 1927.

ROWNTREE, L. G. AND A. M. SNELL. A clinical study of Addison's disease. Mayo Clinic Monographs. Philadelphia, W. B. Saunders Co., 1931.

RUBIN, M. I. AND E. T. KRICK. Proc. Soc. Exp. Biol. and Med. **31**: 228, 1933.

STEWART, G. N. AND J. M. ROGOFF. Proc. Soc. Exp. Biol. and Med. **22**: 394, 1925.

SWINGLE, W. W., J. J. PFIFFNER, H. M. VARS AND W. M. PARKINS. This Journal **108**: 159, 1934.

ZWEMER, R. L. Endocrinol. **18**: 161, 1934.

THE EFFECT OF ANOXEMIA ON THE PYLORIC SPHINCTER[1]

EDWARD J. VAN LIERE, GEORGE CRISLER AND I. A. WILES

From the Department of Physiology, West Virginia University, Morgantown, West Virginia

Received for publication July 16, 1934

In a previous paper (Van Liere, Crisler and Robinson, 1933) it was reported that anoxemia caused a delay in the emptying time of the stomach in normal dogs. It was thought that it would be of distinct interest to study the effect of anoxemia on the pyloric sphincter.

METHODS. Barbitalized dogs were used. The dose of barbital was 200 mgm. per kilo body weight. A tracheal cannula was inserted. An incision was made in the midline of the abdomen, the pylorus was exposed and into it was placed a flexible tube especially made for this purpose. This was connected to a pressure tonometer and a recording device described by Thomas (1929). No further operative procedures were done. Anoxemia was produced by allowing the animal to breathe out of a rubber bag which was connected to an apparatus previously described (Van Liere, 1927) which mixed the oxygen and nitrogen so that any desired percentage of oxygen could be administered. A flutter valve was placed in the otherwise closed system, so that the animal could expire to the outside. This prevented the accumulation of carbon dioxide.

During the control period the animal was allowed to breathe a 20 per cent oxygen mixture, that is, approximately the same amount of oxygen as is found in atmospheric air. When it was desired to administer anoxemia, the amount of oxygen was cut down to the desired percentage without disturbing other factors and the same pressure relations were maintained. The changes in the mixture could be made quickly as there was a large flow of gas. Attention was given, of course, to other details that were essential for the production of an accurately controlled anoxemia and for uniform experimental conditions.

RESULTS. Figure 1 shows the effect of 8 per cent oxygen. The anoxemia caused a marked fall in tone and the height of the pyloric contractions was also diminished. Two or three minutes after anoxemia was discontinued the height of the contractions was increased greatly over the preceding normal—a supernormal phase. In figure 2, 5 per cent oxygen pro-

[1] A part of the expense of this investigation was defrayed by a grant from the Committee on Scientific Research of the American Medical Association.

duced a distinct fall in tone and the pyloric contractions gradually became smaller until they were abolished. About two minutes after the anoxemia had been discontinued the pyloric contractions reappeared. Figure 3 shows a sharp rise in tone; the same animal was used as in figure 2, and the same degree of anoxemia maintained. Figure 4 demonstrates the fact

Fig. 1. Eight per cent oxygen. Anoxemia produced a fall of tone and a diminution in the height of the pyloric contractions.

Fig. 2. Five per cent oxygen. Anoxemia produced a marked fall in tone and the contractions were abolished.

that anoxemia is still effective as a stimulus after both vagi have been cut. This tracing also shows that anoxemia is capable of stimulating the pyloric contractions, that is, as far as the height of the contractions is concerned.

Discussion. It will be seen by looking at the graphs that anoxemia at times produced a rise in the tone of the pyloric sphincter and at other times

a fall. As a rule an animal would give consistent results, that is, anoxemia would produce a fall in the tone of the pylorus each time or a rise in tone as the case might be. In a few animals, however, the responses were not consistent as anoxemia would produce a rise of tone at one time and a fall

Fig. 3. Five per cent oxygen (same animal as in fig. 2). Anoxemia caused a rise in tone. Initial stimulation of the contractions occurred followed by a subsequent depression.

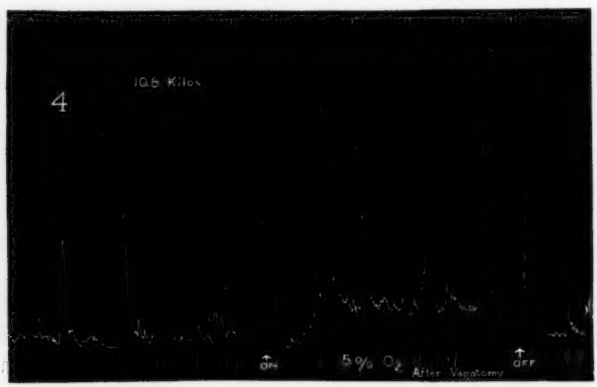


Fig. 4. Shows the effect of 5 per cent oxygen after double vagotomy. There was still a vigorous response.

at another. Figures 2 and 3 demonstrate this. About 25 animals were used and in the majority of cases anoxemia produced a rise in tone of the pyloric sphincter. It was difficult to predict whether anoxemia would cause a rise or fall in pyloric tone, although there was some evidence indi-

cating that if the tone were low, anoxemia might produce a rise while if the tone were high the opposite might be obtained.

In some instances pyloric contractions appeared to be stimulated by anoxemia, particularly at the beginning of the anoxemia period. As a rule, however, after this initial stimulation the contractions became smaller and in many cases with severe grades of anoxemia, completely abolished.

The criticism might be raised that rather severe grades of anoxemia were used. This was found to be necessary. We feel that in unanesthetized animals less severe grades of anoxemia would have been effective. It was found that with barbital anesthesia the threshold in most experiments was about 9 or 10 per cent oxygen. To obtain distinct manifestations of change in tone of the pyloric sphincter, however, or changes in the height of contractions a more severe grade of anoxemia was required.

It was found that ether is not a suitable anesthetic; it itself is capable of stimulating the pyloric sphincter. Furthermore, changes in respiration which accompany anoxemia would effect the depth of ether anesthesia. Sodium barbital worked very well and with the doses used no difficulty was experienced in obtaining suitable control contractions.

It was felt that anoxemia might cause a vagospasm as it does in the case of the heart (Van Liere and Crisler, 1933). This would manifest itself, of course, as a pylorospasm. It was found, however, that after the vagi were cut, the response to anoxemia was not always abolished and in some instances after double vagotomy anoxemia would still produce a vigorous effect. This is clearly shown in figure 4.

It is generally held that epinephrin is given off during anoxemia and it was suggested by Doctor Thomas (personal communication) that in his opinion some of the curves resembled those which may be obtained by the injection of epinephrin. Not all the curves, however, are typical epinephrin curves so this mechanism cannot be used to explain all the tracings.

Besides the question of epinephrin and vagal action, there are several other possible mechanisms which might be mentioned. It is probably possible for anoxemia to influence the intrinsic nervous mechanism of the pylorus or to act directly on the smooth muscle. It is generally conceded that the vagus carries both motor and inhibitory fibers to the pylorus and the same is probably true for the splanchnics (Thomas and Wheelon, 1922; Carlson and Litt, 1924). We are dealing, therefore, with a complicated mechanism. It becomes still more complicated when one considers that anoxemia doubtless can stimulate the splanchnics; epinephrin would then be thrown into the circulation and the pylorus would then be affected indirectly by the anoxemia.

In the light of the complexity of the control of the pyloric sphincter and the general nature of anoxemia as a stimulus, it is not surprising that inconsistent results were obtained.

SUMMARY AND CONCLUSIONS

In a series of barbitalized dogs it was found that anoxemia caused a rise in tone of the pyloric sphincter in some animals and a fall in tone in others. In a few animals anoxemia caused a rise at one time and a fall at another.

As a rule the height of the pyloric contractions was diminished by anoxemia, especially if severe grades were used. In some instances anoxemia produced an initial stimulation, so that the contractions actually were higher, although this was generally followed by a subsequent depression.

This variability in the results we attribute to the complexity of the control of the pylorus and to the general nature of the stimulus of anoxemia.

We take pleasure in expressing our thanks to Dr. J. E. Thomas for the use of his apparatus and for his helpful suggestions.

REFERENCES

CARLSON, A. J. AND S. LITT. Arch. Int. Med. **33**: 281, 1924.
THOMAS, J. E. This Journal **88**: 498, 1929.
THOMAS, J. E. AND H. WHEELON. J. Lab. Clin. Med. **7**: 375, 1922.
VAN LIERE, E. J. This Journal **82**: 727, 1927.
VAN LIERE, E. J. AND G. CRISLER. This Journal **105**: 469, 1933.
VAN LIERE, E. J., G. CRISLER AND D. ROBINSON. Arch. Int. Med. **51**: 796, 1933.

THE RELATION OF STRAY LIGHT IN THE EYE TO THE RETINAL ACTION POTENTIAL

GLENN A. FRY[1] AND S. HOWARD BARTLEY[2]

From the Department of Ophthalmology and Laboratory of Neurophysiology, Oscar Johnson Institute, Washington University, Saint Louis

Received for publication November 14, 1934

Granit (1933) has recently shown that the complex retinal action potential can be attributed to three different processes, PI, PII, and PIII, which in the case of the cat can be isolated by ether and asphyxiation. Ether, according to Granit, removes both PI and PII, whereas asphyxiation only removes PII.

A satisfactory explanation of the nature of these three processes has not as yet been found. One stumbling block in the road to the solution of this problem has been the failure to see that stray light plays an important part in determining the retinal action potential. Previous investigators have simply assumed that the retinal action potential is determined for the most part by the elements in the focal area.[3] But if stray light is at all adequate to stimulate the thousands of photoreceptors in the non-focal area, this area by virtue of its large number of elements might produce a potential of considerable size when that produced by the focal area would not be measurable.

We have devised the following experiment which can be used as a test of whether the focal *b*-wave is of measurable size. The experiment consists in exposing alternately to the eye two bright areas in a dark field, so that one area is covered the moment the other is exposed. Since both produce the same quantity of stray light, and since the stray light in both cases is distributed fairly uniformly over the retina, the alternation from one area to the other should not produce any change in the stray light, but each of the two small retinal spots should respond to its intermittent stimulation.

The apparatus used in this experiment is illustrated in figure 2. The

[1] National Research Fellow in the Biological Sciences.

[2] The experimental work was commenced while the author was a National Research Fellow in the Biological Sciences and completed while he was a beneficiary of a Grant-in-Aid for Research in Neurophysiology, from the Rockefeller Foundation.

[3] We have applied the word, *focal*, to the area of the retina receiving focalized light, the image, and *non-focal* to the rest of the retina which receives only stray light.

screen in front of *Lens I* has two apertures, ½ inch in diameter, and 2 inches apart (center to center). The rabbit's eye was placed 5½ inches from this screen. The disk rotating in front of the condensing lenses cut the illumination of the two apertures on and off alternately. The brightness of the apertures was set at 3100 c. per sq. ft. The electrodes were placed on the cornea and the tissue back of the eye and the response was recorded with a cathode ray oscillograph and a condenser-coupled amplifier.

Figure 3A shows a record of what is obtained when one area alone stimulates the eye at a rate of 2 per second. The waves correspond to the ordinary *b*-waves of the retinal response to a flash of light. If this record

Fig. 1. Showing how the measured latency of the *b*-wave might represent the latency of either the focal or the non-focal response. In each case the dotted-line represents the non-focal component, the dash-line the focal component, and the solid line the total response.

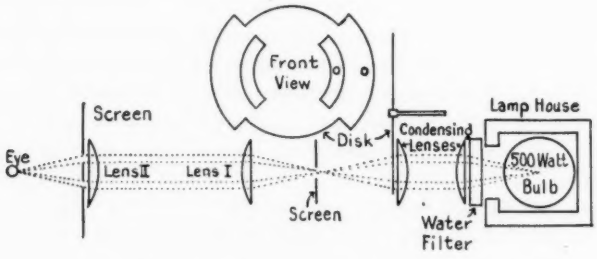


Fig. 2. Apparatus for alternating two stimulus areas. The front view of the disk shows its position with respect to the two beams of light which it intercepts.

represents the response of the focal area, then when the two areas are intermittently exposed out of phase with each other, the summed response for the two focal areas should be as indicated in figure 3B, but under these conditions no response at all is obtained. Since the stray light is kept constant under these conditions, the response in figure 3A must be attributed to the intermittence of the stray light in the non-focal area.

The above finding that the retinal response is determined largely by the stray light in the non-focal area is confirmed by the fact that changes in stimulus area and intensity affect the latency of the *b*-wave in the same way. When we come to interpret the latency of the *b*-wave, we have to consider first of all whether the latency measured is that of the elements in

the focal or the non-focal area. The *b*-wave represents a combination of two waves which for convenience let us call the focal and non-focal *b*-waves. Since the focalized light is much more intense than the stray light, it might be supposed that the focal *b*-wave precedes the non-focal. If the focal *b*-wave were of material size as indicated in figure 1B, the measured latency of the recorded *b*-wave would represent the latency of this wave,

Fig. 3. A. Response from intermittent exposure of one of the stimulus areas at a rate of 2 per second. The on and off phases are equal and are indicated in the time scale by the white and shaded divisions, respectively.

B. Response which should be obtained from alternating the two stimulus areas, provided the response in A represents the response of the focal area. The junctures in the time scale represent the moments of alternation, the frequency of alternation being 4 per second.

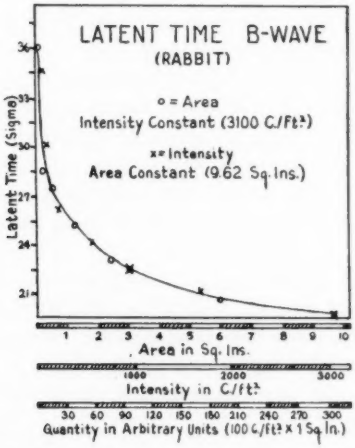


Fig. 4. A comparison of the effects of varying stimulus area and intensity upon the latency of the *b*-wave in the rabbit.

but if the size of the focal *b*-wave were not appreciable as indicated in figure 1A, the onset of the non-focal *b*-wave would probably be the point used in actual experimentation for measuring the latency of the *b*-wave.

We have found that the changes in the latency of the *b*-wave induced by changes in stimulus area can be copied by corresponding changes in intensity. (This is shown in fig. 4.) The responses studied in this connection are responses to stimuli lasting 100 sigma. The grid electrode was placed on the cornea and the ground electrode on the back of the eye. The dis-

tance from the eye to the stimulus was 5½ inches. The general fact that the latency of the *b*-wave varies with stimulus area can be accounted for on two hypotheses: On the one hand it might be assumed, as previous investigators have done, that the *b*-wave represents the response of the focal area, that it represents a post-synaptic process, and that the variation in latency with stimulus area depends upon spatial summation at the synapses. On the other hand, it might be assumed that the measured latency is that of the non-focal *b*-wave. In this case the variation in latency could be attributed to the change in quantity of stray light. The facts fit very nicely into this second hypothesis, for since corresponding reductions in stimulus area and intensity produce corresponding changes in quantity of stray light, the latency of the *b*-wave should depend alike upon stimulus area and intensity and this is exactly what is found to be the case. If the response were that of the focal area, or if the latency of the non-focal response were affected by the focal area, there is no *a priori* reason why changing the size of the focal area, if it produced any effect upon latency at all, should produce exactly the same effect as changing the stimulus intensity. Thus a discrepancy might be expected between the curves for area and intensity, but none occurs.

Adrian and Matthews (1927) have investigated the relation of the *a*-wave to stimulus area and intensity. Although their results for area and intensity are plotted on separate graphs in their paper, namely, C and D in figure 19, page 402, the curves coincide when they are plotted on the same graph, intensity and area values being adjusted to represent equal quantities. This means that the latency of the *a*-wave is affected alike by area and intensity.

If the effects of area upon the latency of the *b*- and *a*-waves can be attributed to the increase in stray light, as the facts seem to justify, i.e., if spatial summation at the retinal synapses is not needed to explain the facts, the present findings furnish no evidence that either PII or PIII is post-synaptic, and either or both might be assigned to the photoreceptor level.

It should be noted that our findings relate only to the action currents recorded from the eye. Bartley (1935) has found discrepancies between area and intensity for the latency of the cortical action currents in the case of the rabbit and Adrian and Matthews (1927) found similar discrepancies for the optic nerve response in the case of the eel. At the optic nerve and cortical levels which are definitely known to be post-synaptic, the non-focal region might be affected by the focal, or the focal region might be represented directly in the action potential. In the case of the retina, the geometry of the eye as well as the small number of elements involved might prevent the focal response from being recorded.

A second test which previous investigators have used for spatial summation as a factor affecting the latency of the *b*-wave, consists in dividing the

focal area into parts and changing their distribution over the retina to see whether the latency of the *b*-wave can be affected in this way. Granit (1933) performed an experiment of this kind and obtained results which led him to conclude that the more concentrated the stimulus area the shorter becomes the latency of the *b*-wave. But there are certain objections to his procedure which affect the validity of his results. He measured the latency obtained with a single disk-shaped area, 6 mm. in diameter, and then with four such disks arranged in the shape of a square, and found them to be 67 ±2.3 sigma and 59 ±2.0 sigma. Thus increasing the area fourfold decreased the latency 11.9 per cent. He then compared the latency for a disk 6 mm. in diameter with that for one 12 mm. in diameter, these latencies being 64 sigma and 54 sigma respectively. The percentage decrease in this case was 15.4 per cent. The fact that different values were obtained for a 6 mm. disk in the two parts of the experiment shows that the condition of the cat's eye changed and the difference in percentage reduction might be a function of this change rather than of the change in distribution of stimulus area. This renders inconclusive Granit's evidence that the latency of the *b*-wave is affected by the distribution of the stimulus area.

We have investigated the effect of varying the distance apart of four small stimulus areas, the total area being kept constant. A series of patterns was designed. One contained a circular aperture $\frac{3}{4}$ inch in diameter. The rest contained four circular apertures $\frac{3}{8}$ inch in diameter, but spaced at different distances apart, and in each case forming the corners of a square. The diagonals of the squares were $\frac{5}{8}$, $\frac{7}{8}$, $1\frac{1}{8}$, $1\frac{3}{8}$, $1\frac{5}{8}$, $2\frac{1}{8}$, $2\frac{5}{8}$, and $3\frac{1}{8}$ inches, i.e., measured from the centers of the apertures. These patterns were placed in front of a bright surface $5\frac{1}{2}$ inches from the rabbit's eye. The stimulus duration was 100 sigma and the intensity was 1900 c. per sq. ft. The grid electrode was placed on the cornea and the ground electrode on the back of the eye. In each case the latency of the *b*-wave for a single spot was measured as well as for all four together. The latency for the former remained constant at 25 sigma and for the latter at 20 sigma. The latency for the single large area ($\frac{3}{4}$ in. in diameter which is equivalent to four areas $\frac{3}{8}$ in. in diameter) was also 20 sigma.

The failure of changing the distribution of the stimulus area to affect the latency of the *b*-wave conforms well to the stray light hypothesis, for according to this the distribution of focal areas should not affect the amount of stray light as long as the total area is constant.

SUMMARY

A method has been described for ascertaining whether the measured latency of the electrical response from the eye represents the latency of the response in the focal area (the area receiving the image) or the non-focal area (the area receiving only stray light) or that of both. The method

consists in alternating two stimulus areas so that one appears when the other disappears. In this way the stray light is held constant and the focal areas have a chance to manifest themselves independently of the non-focal area, but do not do so, the failure of a response indicating that the retinograms record only the non-focal activity.

The changes in the latency of the *b*-wave produced by changing stimulus area correspond to the changes produced by changing intensity. Since both factors affect stray light alike, this finding indicates that the effects of changing the area of the stimulus are produced by changing the quantity of stray light. Adrian and Matthews' results show the same thing for the *a*-wave.

The effect of varying the distance apart of four small areas was investigated. This factor did not affect the measured latency of the *b*-wave.

The general conclusion from our findings is that the effects of varying stimulus area as recorded at the eye provide no evidence that PIII and PII are post-synaptic.

REFERENCES

ADRIAN, E. D. AND R. MATTHEWS. J. Physiol. **63**: 378, 1927.
BARTLEY, S. H. This Journal **110**: 666, 1935.
GRANIT, R. J. Physiol. **67**: 207, 1933.

PHYSIOLOGICAL EFFECTS OF PITUITARY GROWTH HORMONE: GROWTH AND EFFICIENCY OF FOOD UTILIZATION[1]

HUGO W. NILSON, L. S. PALMER AND CORNELIA KENNEDY

From the Division of Agricultural Biochemistry, University of Minnesota, St. Paul

Received for publication October 24, 1934

Although Morris, Palmer and Kennedy (1933) found that litter brothers or sisters of stock inbred for more than ten generations are not entirely equals insofar as inherent growth potential and efficiency of food utilization are concerned; nevertheless, several strains of rats have been segregated which differ from one another by approximately 40 per cent in efficiency of food utilization and which show distinct genetic segregation in other respects. It was the purpose of the experiments herein reported to employ animals from these tested strains in an attempt to isolate the effect of the growth hormone from the anterior pituitary on the growth rate and efficiency of food utilization.

Numerous experiments have been reported in the literature proving rather definitely that the growth hormone preparations do affect the rate of growth of individual animals within limits imposed by sex and age, but in only two studies have the diets fed received more than casual mention (Bryan and Gaiser, 1932; Lee and Schaffer, 1934). In the first instance, no attempt was made to correlate growth rate with food intake and in the second case the use of the paired feeding method may be criticized since litter mate controls do not necessarily possess genetic equality with the treated animals and uniform food intake is no assurance of equal food utilization.

Allotment and care of animals. The "high efficiency" and "low efficiency" strains of hooded rats had been established by inbreeding from 8 to 15 generations and represented respectively rapid and slow growing strains which also differ by about 40 per cent in levels of efficiency of food utilization. The albino "line 8" strain approximated the "high efficiency" strain in these respects. The three strains had the same paternal ancestral

[1] The data were submitted by Hugo W. Nilson to the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Doctor of Philosophy, June, 1934. All pertinent individual data are available for reference from the Division of Agricultural Biochemistry. Paper no. 1315, Journal Series, Minnesota Agricultural Experiment Station.

background, and the maternal ancestors of the hooded versus the albinos differed only by two outcrosses made in 1922.

All animals were allotted to experiment when they reached 60 grams initial live weight. They were individually housed in galvanized iron screen cages (12 x 12 x 10 inches) fitted with screen floors, at an environmental temperature of 81°F. The basal diet, fed *ad libitum*, consisted of commercial casein, 35; pearl tapioca dextrin, 37; lard, 15; butterfat, 9 and Hawk and Oser (1931) salt mixture, 4 parts by weight. The daily supplemental diet consisted of 0.5 gram of frozen beef liver, 0.25 gram of dried yeast and 0.95 gram of commercially dried lettuce. Distilled water consumption was recorded.

All rats were maintained for three weeks on the basic environmental régime (control period) in order to determine the normal rate of growth by the method of Brody (1927) and the coincident efficiency of food utilization as measured by the Palmer and Kennedy (1931) efficiency quotient. A second three-weeks period immediately followed the first in which animals selected approximately at random were injected daily with growth hormone preparation, while others were continued as check (non-injected) animals. In this way each injected animal served as its own control and litter mates or animals of like degree of inbreeding served as additional checks.

The growth hormone preparations. A few animals were treated with Phyone while the remainder were injected intraperitoneally with antuitrin G which was kindly supplied by Parke, Davis & Co., Detroit. With few exceptions, each treated animal was arbitrarily injected with 1 cc. daily. Quantitative assays of the hormone preparations were not thought possible but it was hoped that the limit of effect permitted by environment would be produced by this level of injection in at least one group.

Growth studies. The growth data (fig. 1) plotted on semi-log scale indicate that the growth rate during the first three-weeks control period was remarkably similar for comparable groups, but during the second three-weeks period only the injected and check "high efficiency" male groups showed comparable growth rates. The injected animals outgrew the checks in the order of "low efficiency" male, "high efficiency" female and "low efficiency" female groups.

Brody (1927) found that after puberty the growth rate is no longer a direct function of the inherent growth potential, but the rate decreases due to an increasing concentration of growth inhibitors. His method of graphical analysis is used in figure 2 to determine the effect of the growth hormone and the genetically controlled factors on the growth rate. The continuous lines represent the data plotted on the basis of an interpolation of mature weight from the figure 1 growth curves for the first three-weeks control period, and the short lines represent a re-interpolation for the

second three-weeks period when the first curves show distinctly by a downward trend that a change in growth rate has occurred.

This analysis shows definitely that the growth hormone preparations did not affect the growth rate of the comparatively rapid growing "high efficiency" and "line 8" male groups. But they did stimulate in increasing

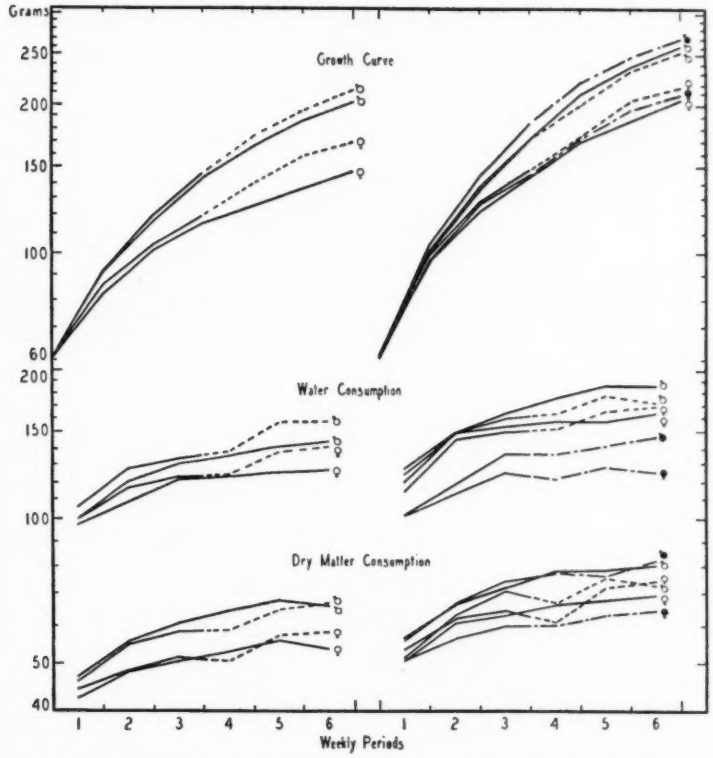


Fig. 1. Growth, dry matter and water consumption data by weekly periods. The curves of the low efficiency strain are on the left side, while those of the high efficiency and line 8 (filled sex symbols) strains are on the right side of the graph. The broken lines indicate injection periods and the solid lines indicate control periods.

order the growth of the "low efficiency" males, "line 8" females, "high efficiency" females and the "low efficiency" females. This analysis is entirely dependent upon the *same* individuals supplying both the control and treatment data. The data also show that the percentage growth rates of all the check (non-injected) groups except the "low efficiency"

female group remained constant during the entire six-weeks period. The exception is at present unexplainable. Parenthetically, the short vertical lines show the calculated age when the post puberal growth rate begins. The more rapidly growing strains enjoy a longer period of unhindered prepuberal growth.

This graphical analysis should clearly evaluate the growth statistics if homozygous strains of rats were used, but the data in table 1 show that

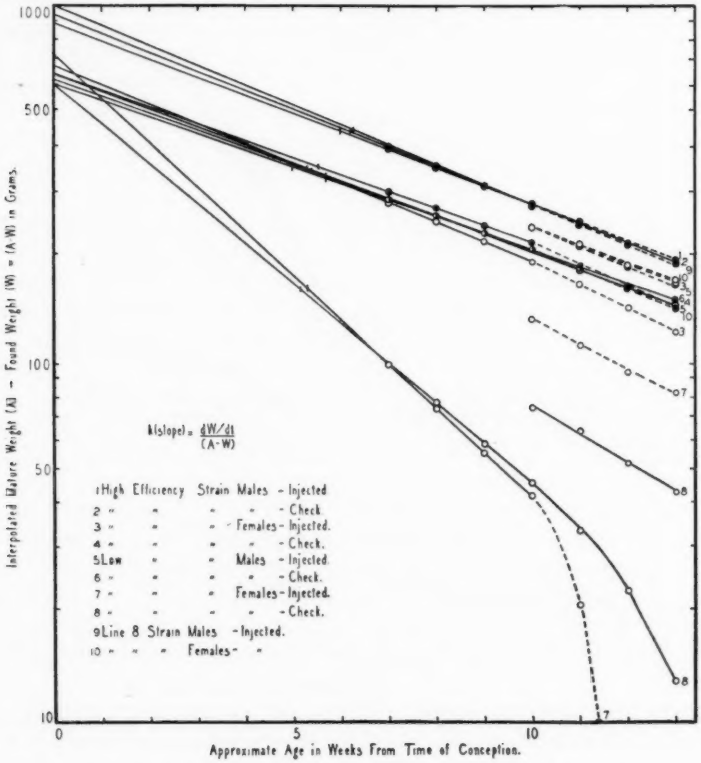


Fig. 2. Graphical analysis of group growth curve data

most of the group means used in plotting the curves of figure 2 represent values which are not sufficiently accurate for interpretation by Brody's method.

Only the correlation coefficients of the "line 8" males and of the injected and check "high efficiency" female groups indicate a reasonable fixity of purpose and may be considered within the 5 per cent level of significance (Wallace and Snedecor, 1931). Nevertheless, the trends empha-

sized by the Brody type of analysis apparently did take place within the limits imposed by rather heterozygous experimental subjects and a possible individual variation in response to hormone. Furthermore, specific body measurements and superficial autopsy studies indicated that the growth of the injected rats was normal.

Dry matter and water consumption studies. The curves in figure 1 show rather marked and strikingly regular effects of injection on both the dry matter and water consumption. However, these differences have no effect

TABLE 1

Statistical analysis of variations in gains in weight by groups during the two three-week periods

GROUPS	n	FIRST		SECOND		r
		Aver. gain	C.V.	Aver. gain	C.V.	
		grams	*per cent*	*grams*	*per cent*	
H. E. males	Injected 14	109.2	6.53	84.5	12.20	−0.092
H. E. males	Check 14	109.3	6.95	88.4	12.24	+0.383
L. 8 males	Injected 8	121.8	8.04	87.1	25.22	+0.842
H. E. females	Injected 20	87.0	13.25	68.8	18.25	+0.706
H. E. females	Check 20	83.4	8.88	55.8	21.33	+0.691
L. 8 females	Injected 9	82.6	20.34	64.7	23.51	+0.398
L. E. males	Injected 35	85.1	18.34	67.1	19.11	+0.292
L. E. males	Check 35	80.1	12.65	61.4	14.63	+0.284
L. E. females	Injected 37	57.6	10.71	50.5	25.50	+0.032
L. E. females	Check 31	53.8	12.96	33.4	23.50	−0.124

n = number of animals in group

C.V. (coefficient of variation) $= 100 \dfrac{\sqrt{\dfrac{S(X)^2 - S(X)\,\bar{X}}{n-1}}}{\text{mean}}$

r (coefficient of correlation) $= \dfrac{\dfrac{S(XY) - S(X)\,\bar{Y}}{n-1}}{\sigma_x \times \sigma_y}$

on the gain in weight as indicated by the regularity of the growth curves. A statistical analysis shows that significant correlation coefficients between dry matter intake of the first and second three-weeks periods were obtained in all cases, and comparable groups consumed approximately the same total quantity of dry matter. Significant differences were only obtained for the "high efficiency" males with differences in grams of 9.95 $t = 2.23$ and 21.25 $t = 3.27$ respectively for the first and second three-weeks period.

The water consumption curves show a remarkable constancy in the relationship between water and dry matter intake; the hormone did not exert any tendency to increase the water consumption contrary to the assumption of Downs (1931). The "line 8" strain consumed less water in relation to either gain or dry matter consumption than did the other two strains.

Efficiency quotient studies. Palmer and Kennedy (1931) promulgated an efficiency quotient (E.Q.) in respect to dry matter according to the equation

$$\frac{\text{dry matter consumed, grams}}{\text{gain in live weight, grams}} \div \frac{\text{mean live weight per period, grams}}{100}$$

This correlates the data on gain in weight and dry matter consumption into a single index representing the efficiency of food utilization. In figure 3 the mean E.Q. for the first three-weeks control period was lowest for the "line 8" males which means that these animals were the more efficient in producing gain in weight. The two "high efficiency" male groups followed closely and there is little to choose between the mean efficiency of the three female groups. The comparative efficiency is in the order of 100:92:88:68:67:66 per cent. During the second three-weeks period, the comparative efficiency may be roughly scored as 100:100:100:73:78:57 per cent respectively. This means that the injected females were more efficient than expected.

Similarly in figure 4 an analysis shows the comparative efficiency during the first three-weeks control period to be 100 ("line 8" males as a standard) 72:67:47:44 per cent and for the second three-weeks period, 100: 75:66:52:32 per cent respectively. The injected low efficiency groups did better than was expected. More noteworthy is the fact that the injected "low efficiency" female as well as the two high efficiency female groups behaved more like males since they increased their efficiency of utilizing dry matter for growth.

The statistical analysis of variation within groups (table 2) for the first time indicates significant trends between comparable injected and check groups. The correlation coefficient for the injected "low efficiency" female group is numerically significant while that for the check group is not. This difference is noted in spite of the skewed distribution of the injected animals (fig. 4) which is probably due to the exceptional potency of lot 095176B of antuitrin G.

Physiological studies. Table 3 presents certain physiological data which show that differences in live weight may be used as an index of true gain in weight, at least when the animals are fed an identical dietary. They also show rather definitely that the injection technique was not disturbing

since the apparent digestibility of dry matter was not affected. Nor can differences in level of efficiency of food utilization be explained on these

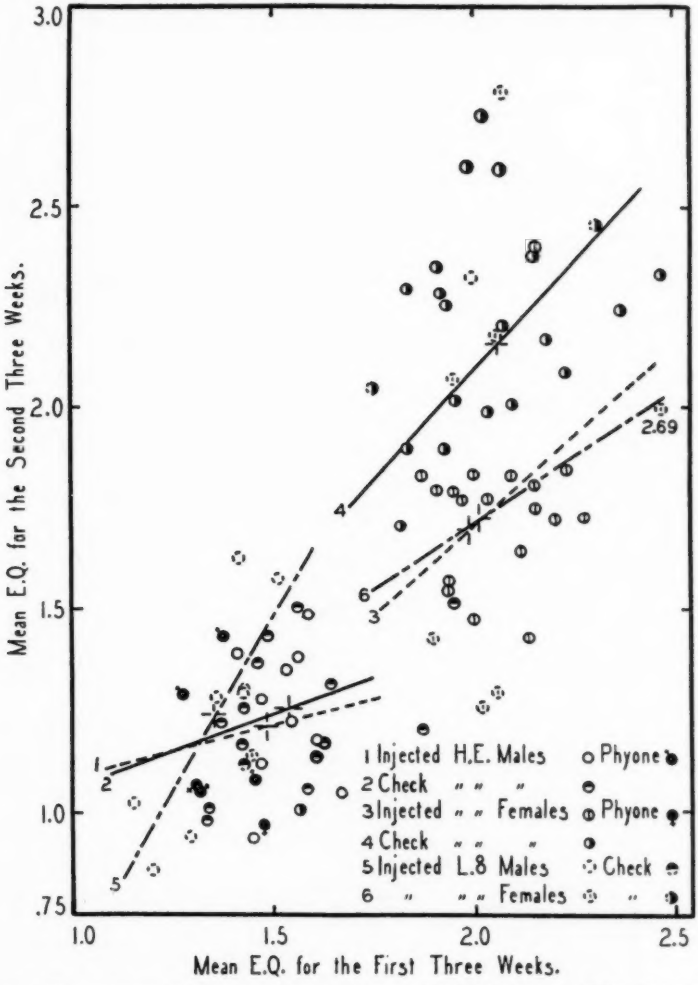


Fig. 3. Dry matter efficiency quotient studies. High efficiency and line 8 strains

grounds. The chemical analyses show that the males are more efficient in utilizing food in part because the energy of "synthesis" per unit increase in weight is less due to the lower dry matter and fat content, and the

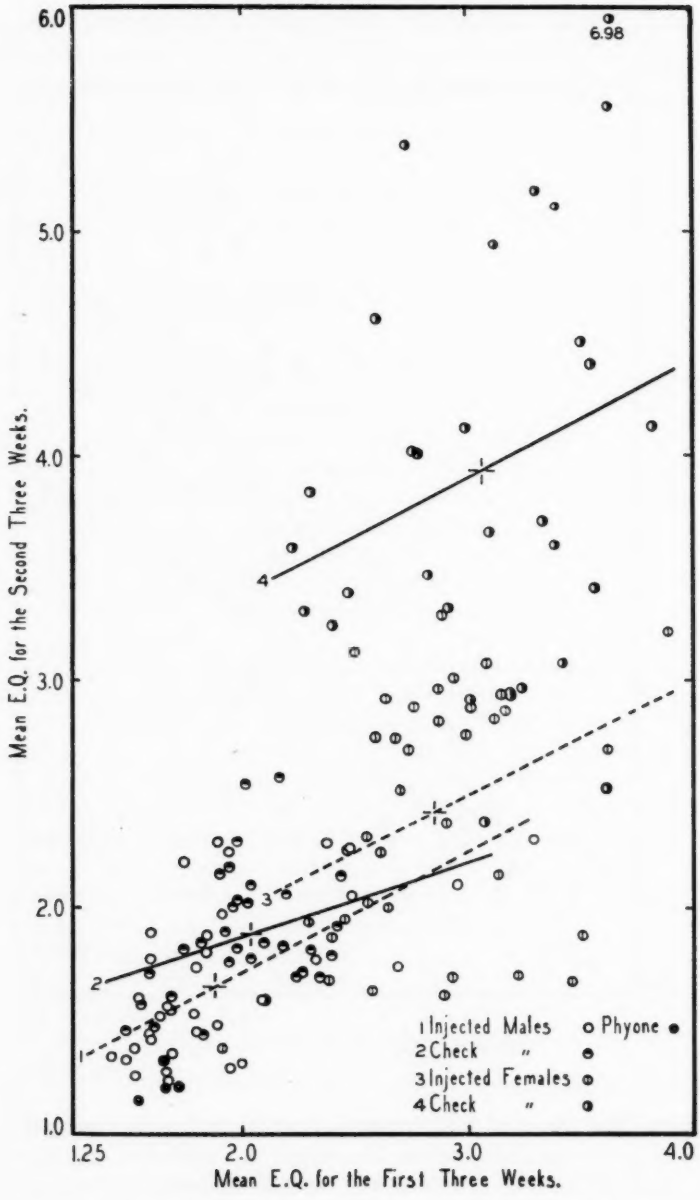


Fig. 4. Dry matter efficiency quotient studies. Low efficiency strain

TABLE 2

Statistical analysis of variations in dry matter efficiency quotients by groups during the two three-week periods

GROUPS	n	FIRST		SECOND		r
		Aver. E.Q.	C.V.	Aver. E.Q.	C.V.	
			per cent		*per cent*	+
H. E. males	Injected 14	1.47	7.61	1.21	13.43	0.175
H. E. males	Check 14	1.54	12.11	1.25	13.79	0.421
L. 8 males	Injected 8	1.35	8.86	1.24	21.40	0.778
H. E. females	Injected 20	1.99	11.82	1.70	17.61	0.710
H. E. females	Check 20	2.05	10.20	2.17	17.10	0.610
L. 8 females	Injected 9	2.01	15.87	1.73	25.92	0.460
L. E. males	Injected 35	1.88	22.99	1.65	22.96	0.631
L. E. males	Check 35	2.03	13.22	1.89	14.77	0.355
L. E. females	Injected 37	2.84	14.20	2.41	23.26	0.359
L. E. females	Check 31	3.08	14.55	3.93	25.26	0.249

TABLE 3

Summary studies on the relationship of empty carcass weight to live weight, apparent dry matter digestibility coefficients for the 2nd and 5th weeks, and the chemical composition of the empty carcasses for selected groups

GROUPS		EMPTY CAR. TO L. W.	APP. D.M. DIGEST COEF.		DRY MATTER	PROTEIN	FAT	ASH
			2nd week	5th week				
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
H. E. males	Injected	96.3	94.0	93.8	36.2	19.3	12.2	0.03
H. E. males	Check	97.2	93.5	93.2	35.9	19.5	10.6	0.03
L. 8 males	Injected	97.2	94.9	94.2	38.8			
H. E. females	Injected	96.3	93.5	93.2	38.9	18.1	15.5	0.03
H. E. females	Check	96.3	94.1	93.7	38.9	19.3	14.5	0.03
L. 8 females	Injected	96.2	94.2	94.4	38.6			
L. E. males	Injected	96.3	93.5	94.4	34.8	20.3	9.4	0.03
L. E. males	Check	96.7	93.7	93.7	34.7	19.3	10.6	0.03
L. E. females	Injected	95.3	93.4	93.7	33.8*	19.1	9.4	0.03
L. E. females	Check	96.1	93.8	93.4	39.5*	19.3	14.7	0.03

* Difference in grams 5.72 t = 5.598 (t = diff./S.E. diff.)

hormone, particularly in the limiting case, tends to change the composition of the tissues to the more easily synthesized type. Note particularly the hydration of tissues of the injected "low efficiency" females.

Discussion. Apparently, there is a very close and direct relationship between growth and the efficiency of food utilization according to the weekly period changes and this trend is emphasized by the graphical analysis of group growth rate data. However, the statistical evaluation of mean group weights indicates that the variation in the check (non-injected) groups is probably due to the imperfect expression of genetic control, and though this factor is reduced in the injected groups there is on the other hand an increased variability due to variable individual response; variable potency of the hormone preparation, and variable dosage caused by leakage through the hypodermic needle hole probably should be considered a minor factor. Also, the unit of hormone preparation used may not always have been sufficient to produce maximum effects in all groups.

The same errors of analysis also hold for the interpretation of dry matter intake data but are less important because fewer variables control food intake. The detailed data on coefficient of apparent digestibility, voluntary activity, regularity of estrous cycle and metabolic level none of which are included in this paper show these to be unimportant factors affecting dry matter consumption. Furthermore, the controlled environmental temperature reduced this variant to a minimum. Consequently, the correlation coefficients comparing the dry matter intake of the second with the first three-weeks period of both the injected and non-injected (check) animals may be expected to be rather significant and the coefficients of variation should be correspondingly small.

These relationships indicate that there need to be no perfect correlation between growth rate and food consumption; consequently, it is entirely possible that the animals with higher growth rates consume relatively less food. If the growth hormone preparations promote a higher level of efficiency one should expect the relative significance of the correlation coefficients between efficiency during the second and the first three-weeks period for both injected and check animals to be about the same as those for the trend of effect on growth rate. This relationship was noticeable even to the extent of weekly period changes except that the dry matter and water consumption data indicate that there is a bare possibility the initial effect of the growth hormone preparation was on the hydration of tissues.

CONCLUSIONS

1. Pituitary growth hormone preparations apparently promote growth and increase the efficiency of dry matter utilization within certain limits set by the inherent level which appears to be under genetic control and sex.
2. The female rats are affected to a rather marked extent by the pitui-

tary growth hormone preparations towards the attainment of a male ideal in growth rate and efficiency of dry matter utilization. Strains of rats with a lower order of efficiency are affected less definitely towards the attainment of a higher efficiency level.

3. Water consumption is apparently closely correlated with dry matter intake and the injection of a pituitary growth hormone preparation into rats does not increase their relative water consumption.

4. The differences in the efficiency of dry matter utilization by rats cannot be explained by differences expressed as apparent dry matter digestibility coefficients, either insofar as inherent levels or hormone effect are concerned. The injection operation does not affect the level of apparent digestibility.

5. Changes in live weight of rats are apparently satisfactory indices for true growth of this species as represented by probable changes in empty carcass weight.

6. There are definite indications that there is a decrease in dry matter and fat content of the empty carcasses of female rats having low efficiency of dry matter utilization following injection of pituitary growth hormone preparations.

REFERENCES

BRODY, S. Univ. of Mo. Agric. Exp. Sta. Res. Bul. **97**: 58, 1927.
BRYAN, A. H. AND D. W. GAISER. This Journal **99**: 379, 1932.
DOWNS, W. G., JR. Arch. Path. **12**: 37, 1931.
HAWK, P. B. AND B. L. OSER. Science **74**: 369, 1931.
LEE, M. O. AND N. K. SCHAFFER J. Nutr. **7**: 337, 1934.
MORRIS, H. P., L. S. PALMER AND C. KENNEDY. Univ. Minn. Agric. Expt. Sta. Tech. Bul. **92**: 1, 1933.
PALMER, L. S. AND C. KENNEDY. J. Biol. Chem. **90**: 545, 1931.
WALLACE, H. A. AND G. W. SNEDECOR. Iowa State College Official Publication **30**: 62, 1931.

PROLACTIN INDUCES BROODINESS IN FOWL[1]

OSCAR RIDDLE, ROBERT W. BATES AND ERNEST L. LAHR

From the Carnegie Institution, Station for Experimental Evolution, Cold Spring Harbor, N. Y.

Received for publication November 23, 1934

We have previously shown that a distinct anterior pituitary hormone, prolactin, initiates lactation in fully developed mammary glands, induces the growth and functioning of the crop-glands in pigeons (1, 2), and acts adversely on the gonads of the dove, pigeon and fowl (3, 4). The present study demonstrates that this same hormone also induces broodiness in fowl.

Since the growth response in the crop-glands of pigeons is normally associated with broodiness—or at least with the latter part of the broody period—it seemed probable that broodiness in pigeons is initiated by prolactin; but factors contributory to a decisive expression of broody behavior are readily deranged in pigeons, and it was found best to utilize the domestic fowl for this study of the relation of prolactin to broodiness. Moreover, the use of fowl would perhaps permit us to learn something further of the rôle of prolactin in those great groups of vertebrates which can give neither the lactation nor the crop-gland response. The results of this and a simultaneously conducted study (4) demonstrate that the domestic hen responds to prolactin by rapid regression of her ovary, and by broody behavior.

Hens exhibit broodiness—even under definite attempts to discourage it—both by clucking and by persistent incubation or "nesting." This broodiness is normally expressed only at or near the end of a period of egg-laying, and periods of egg production usually persist for many weeks even in hens belonging to "broody races." In a so-called "non-broody race" (e.g., the American White Leghorn) a genetic factor essential to broodiness has been eliminated from most individuals of the race by selective breeding; such individuals give only a partial broody response to prolactin. Some hens of "broody" races also lack the genetic factor for broodiness. In conformity with these facts we find that most laying hens belonging to broody races can quickly be made completely broody by repeated injections of prolactin, and apparently by this anterior pituitary hormone only.

MATERIAL AND METHODS. Most tests on non-laying fowl were made on birds not too closely confined in our own colony houses. Most of the tests

[1] Aided by a grant from the Carnegie Corporation of New York.

on actively laying fowl were made at a neighboring poultry farm and thus their transportation shortly before the beginning of dosage was avoided. The birds were trap-nested. Hens of three broody races and of one non-broody race were studied while in both laying and non-laying condition; their ages ranged from $1\frac{1}{2}$ to $3\frac{1}{2}$ years. Some birds were maintained as controls (4), but an adequate control for results obtained with prolactin is provided by tabulated data from birds injected with other hormones.

The preparations of prolactin, with the exception of that given to two birds (A and B), were free or practically free from follicle-stimulating (immature dove testis test) and thyreotropic (dove thyroid test) hormones. Some preparations were heated to 100°C. for 1 hour to assure destruction of growth hormone, but no "growth" hormone preparations even reasonably free from prolactin were available for direct tests of the relation of a "growth" principle to broodiness. Separate tests, with negative results, were made with follicle-stimulating (F.S.H.) and with thyroid-stimulating preparations or with their combination—all of which were free or practically free from prolactin. The F.S.H. preparations chiefly used were made from the anterior pituitary by methods we have previously described (2). In no case at present can the thyreotropic factor be obtained free from the F.S.H. It is practically certain that the F.S.H. and thyreotropic preparations used also contained the "luteinizing" pituitary factor. Also utilized and found not responsible for broodiness were: The luteinizing factor, as this is obtained from pregnant urine (Prolan); F.S.H. from serum of the pregnant mare (free from thyreotropic and prolactin); estrin (amniotin); and corpus luteum hormone (proluton).

The number of units of prolactin and other pituitary principles injected (intramuscularly) daily varied greatly, and the term of daily (or twice daily) injections varied from 1 day (one case, prolactin) to 20 days. Many birds were killed after 5 to 20 days of dosage in order to learn the effect of the several pituitary principles on the adult ovary of the fowl. The results of that study are presented in an accompanying communication (4).

We are much indebted to the Schering-Kahlbaum Co. and the Schering Corporation for the thyreotropic hormone and proluton used by us; to the Parke, Davis Research Laboratories for mare serum; to the I. G. Farbengesellschaft, Elberfeld, Germany, for prolan; and to E. R. Squibb and Sons for supplies of amniotin (estrin) suitably prepared in corn oil.

RESULTS. *Laying hens treated with prolactin.* Essential details of data obtained on laying hens injected with prolactin are given in table 1. Clucking was induced—usually at 2 to 4 days after first injection—in all of the 20 hens of broody races which were given more than a single injection; and in nearly all cases (nos. 77, 6, 65 and 138 excepted) this was followed (12–24 hours later) and accompanied by more or less persistent nesting (incubation) of eggs.

TABLE 1

The induction of broodiness by prolactin in laying hens of various breeds

NUMBER OF BIRD	BREED OF FOWL	DOSAGE IN BIRD UNITS PER DAY	DAYS FROM FIRST INJECTION TO: Day of last egg	Day clucking began	Day nesting began	Last injection	DISPOSAL OF BIRD AND REMARKS
			Tests made in August				
47	Wh. Rock	20	2	1	3	5	Killed 5th day, nesting
7	Wh. Rock	20	1	3	3	5	Killed 5th day, nesting
81	Wh. Rock	20	4	3	3	9	Killed 9th day, nesting
58	Wh. Rock	20	4	3	3	11	Killed 11th day, nesting
50	Wh. Rock	20	1	3	3	7	Reared chick normally
32	Wh. Rock	20	2	3	3	11*	Reared chicks not well*
69	Wh. Rock	40**	(+)†	—	—	0	Apparently no effect**
			Tests made end of November				
5	Wh. Rock	20	6	2	2	7	Killed 7th day, nesting
44	Wh. Rock	20	4	4	4	7	Killed 7th day, nesting
77	Wh. Rock	20	1	7	—	13	Killed 13th day, clucking
65	R. I. Red	10‡	6	4	—	13	Killed 14th day, clucking
64	Wh. Leghorn	20	?[b]	4	—	8	Killed 8th day, clucked 1 day
3	Wh. Leghorn	20	6	—	—	13	Killed 13th day
46	Wh. Leghorn	20	2	—	—	13	Killed 13th day
			Tests made end of September				
67	Wh. Leghorn	4‡	3[b]	—	—	16	Killed 16th day
91	Wh. Leghorn	4‡	4[b]	4	—	18	Killed 18th day, clucking
			Tests made in May				
30	Wh. Leghorn	60‡	3	3	3½	8	Yg. given 11th day and reared
1	Wh. Leghorn	60‡	6?[b]	3	—	8	Laid 11 days after last dosage
60	Wh. Leghorn	60‡	6?[b]	3	—	8	Laid 18 days after last dosage
184	Eng. Wh. Leghorn	120‡	1	2	3	8	Laid 23 days after last dosage
			Tests made in June				
A	Plym. Rock	20	(+)†	3	3	3	Nested 2 days; laying continued
B	Plym. Rock	20	0	4	4	5	Test ended 10th day, nesting
147	♂ Buff Cochin	60‡	♂	5	—	10	Killed 10th day, clucking, sick
146	♂ Buff Cochin	60‡	♂	4	—	10	Clucked only 2 or 3 days§

TABLE 1—*Concluded*

NUMBER OF BIRD	BREED OF FOWL	DOSAGE IN BIRD UNITS PER DAY	DAYS FROM FIRST INJECTION TO: Day of last egg	Day clucking began	Day nesting began	Last injection	DISPOSAL OF BIRD AND REMARKS
		Tests made March–April					
25	R. I. Red	100	1	3	4	6	Nested 7 days only
150	R. I. Red	20	3	3	4	6	Nested 1 day only
85	R. I. Red	20	1	4	4	6	Nested 1 day only
6	R. I. Red	20	1	4	—	6	Laid 14 days after last dosage
88	R. I. Red	10	3	4	4	6	Nested 2 days only
		Tests made in October					
93	R. I. Red	25‡	2	3	6	10	Nested 1 day only
138	R. I. Red	25‡	0	3	—	10	Killed 10th day

* Beginning with 11th day after first prolactin the injection of follicle-stimulating hormone (20 mgm. daily for 22 days) was substituted in an attempt to end broodiness.

** A single injection only.

† Laying was not interrupted.

‡ This daily dosage administered in two separate injections.

[b] No eggs laid during injections; the figure here indicates time (days) before injection at which last egg was laid.

§ This test was repeated 6 weeks later with an entirely negative result.

Two of the broody birds (nos. 50 and 32) were permitted to hatch eggs and rear young. No. 50 accomplished this in a normal and satisfactory manner. In the case of no. 32 an attempt was made to interrupt broodiness after the eleventh day by injection of F.S.H. for a period of 22 days; broodiness was not thus terminated, but in this case the young chicks were poorly attended after hatching. One hen (no. 30) of the non-broody race promptly showed both clucking and nesting behavior after 3 days, and on the eleventh day was given young ducklings which she firmly adopted and reared. Since Warren (5), in a study of broodiness in two lots of White Leghorns (843 hens), found 4 per cent of broody fowl in one lot and 15 per cent in another, it seems probable that our one positive result with this race was obtained on a potentially broody individual. Four repeatedly injected hens of broody races became only partially broody (no nesting) and these are probably to be regarded as "non-broody" individuals of a broody race. Two roosters of a broody race given high dosage were made to cluck at the same period at which hens begin to cluck, but they showed no interest in the eggs and nests provided for them.

TABLE 2

Pituitary hormones other than prolactin fail to induce broodiness in laying hens

NUMBER OF BIRD	BREED OF FOWL	DAILY DOSAGE	DAYS FROM FIRST INJECTION TO: Day of last egg	Day clucking began	Day nesting began	Last injection	DISPOSAL OF BIRD OR REMARKS
Follicle-stimulating (+ thyreotropic). Tests made in August							
		mgm.					
66	Wh. Rock	20	5	—	—	11	Killed on 11th day
77	Wh. Rock	4	3	—	—	11	Killed on 11th day
88	Wh. Rock	4	1	—	—	14	Killed on 14th day
85	Wh. Rock	8*	1	—	—	9	Killed on 9th day
21G	Wh. Rock	20	1	—	—	9	Killed on 9th day
Tests made in July							
142	Wh. Leghorn	4*	1	—	—	13	Observed to 20th day
99	Wh. Leghorn	4*	1	—	—	13	Observed to 20th day
3	Wh. Leghorn	4*	0	—	—	10	Observed to 20th day
94	Wh. Leghorn	4*	1	—	—	10	Observed to 20th day
97	Wh. Leghorn	4*	1	—	—	10	Observed to 20th day
7B	Wh. Leghorn	4*	1	—	—	10	Observed to 20th day
144	Wh. Leghorn	4*	1	—	—	4	Observed to 10th day
8	Wh. Leghorn	4*	1	6	—	4	Clucked for 70 days (lame)
96	Wh. Leghorn	4*	1	—	—	4	Observed for 30 days
Tests made in December							
79	R. I. Red	4*	0	—	—	9	Killed on 9th day
80	R. I. Red	4*	0	—	—	8	Killed on 8th day
98	R. I. Red	4*	0	—	—	8	Killed on 8th day
Thyreotropic (+ F.S.H.). Tests made September–October							
160	R. I. Red	100**	(+)†	—	—	6	Killed on 7th day
167	R. I. Red	100**	(+)†	—	—	6	Killed on 7th day
80	R. I. Red	400**	(+)†	—	—	3	Killed on 8th day
Mare serum. Test made in October							
206	R. I. Red	4‡	2	—	—	5½	Killed on 8th day
Prolan (Elberfeld). Tests made in October							
1B	R. I. Red	300[b]	1	—	—	10	Killed on 10th day
5B	R. I. Red	300	0	6	—	10	Killed on 10th day

TABLE 2—*Concluded*

NUMBER OF BIRD	BREED OF FOWL	DAILY DOSAGE	DAYS FROM FIRST INJECTION TO: Day of last egg	Day clucking began	Day nesting began	Last injection	DISPOSAL OF BIRD OR REMARKS
Corpus luteum (proluton). Tests made in October							
55	R. I. Red	2§	0	—	—	5½	Killed on 8th day
79A	R. I. Red	2	3	—	—	5½	Killed on 8th day
Estrin (amniotin). Tests made in August							
4	R. I. Red	200¶	(+)†	12	13	16	Accepted and reared chicks
8	R. I. Red	200	(+)†	—	—	6	Observed to 16th day

* Dosage administered in two separate injections daily.
** Guinea pig units (20 units per mgm.), in twice daily dosage.
† Egg laying was not interrupted.
‡ Four cubic centimeters daily in twice daily dosage.
ᵇ Rat units in twice daily dosage.
§ Clauberg units in twice daily dosage (5 Clauberg units = 1 Corner-Allen unit).
¶ Rat units in twice daily dosage; dosage doubled on sixth day.

Laying hens treated with other hormones. Data are given in table 2. F.S.H. (+ thyreotropic) and thyreotropic hormone (+ F.S.H.) were shown to be incapable of eliciting either clucking or nesting behavior in laying fowl, although the former preparation stopped egg laying by the fifth day as in the case of prolactin. Estrin and corpus luteum were also ineffective. The three partial or apparent responses obtained (all at unusual periods after first injection) under dosage with three of the above-mentioned hormones are doubtless to be regarded as spontaneously occurring cases not induced by dosage. Corpus luteum hormone stopped egg laying and definitely inhibited the growth of ova but induced no sign of broodiness.

Non-laying hens treated with prolactin. Prolactin, 10 to 60 units (mostly twice) daily, was injected during 7 to 20 days into 15 Rhode Island Reds, 5 White Wyandottes, and 10 White Leghorns—all in reproductive rest. Nearly all of these birds partially responded by clucking, but none showed nesting behavior. Tabulation of these data is omitted. It is certain that prolactin elicits a partial broody response, and a partial response only, in fowl whose ovaries are already inactive at the initiation of dosage.

Non-laying hens treated with other hormones. F.S.H. (+ thyreotropic) was injected for 8 to 18 days in 15 Rhode Island Reds, 6 White Wyan-

dottes and 10 White Leghorns. The dosage (4 mgm. daily, mostly in twice daily injections) markedly stimulated the ovaries in all cases, but neither clucking nor nesting behavior was obtained in any case. These consistently negative results are not tabulated.

DISCUSSION. There seems to be no previous record of the induction of broodiness by anterior pituitary substance—not even by the unfractionated preparations used by several investigators on laying fowl. The frequent though not quite exclusive use of non-broody races (e.g., White Leghorn) in such studies perhaps supplies a partial explanation of failure to observe broodiness in fowl repeatedly injected with crude preparations. It is possible, however, that the simultaneous administration of other pituitary hormones partly or wholly blocks the broody response that would otherwise be obtained from small or moderate amounts of relatively pure prolactin. We have made too few tests of this point. Our first statement on the result fully presented here was made on June 11, 1934 (6).

Others have previously shown that injections of anterior pituitary substance interrupts ovulation and egg-laying in fowl. Walker (7) clearly showed that an unfractionated aqueous extract and suspension inhibits ovulation with accompanying atresia of numerous ova. This inhibition was confirmed by Noether, who in later reports (8, 9) further concluded that the quantity of substance required to stop ovulation in fowl can be used for the quantitative assay of the ovulation-inhibiting substance—and that the thyreotropic factor is that substance. More recent data already cited, particularly the presence of notable amounts of F.S.H. in the "thyreotropic" preparations used by Noether (and by us in this study), require a revision of his conclusions. Unfortunately, the lack of pure preparations of the thyreotropic principle, more especially its known contamination with F.S.H., precludes definite knowledge concerning its action (presumably through the thyroid) on the ovary. When thyroid substance or thyroxin has been administered to birds (by others to fowl and by us to pigeons) in moderate or physiological dosage an inhibition of ovulation has not resulted.

Though prolactin, as administered in this study, may cause the laying hen to become at least partially or temporarily broody *prior* to the time she ceases to mature, ovulate and lay eggs (see table 1) it is clear that complete and persistent broodiness ensues only when the quantity and duration of dosage has markedly repressed her ovary and effectively stopped the growth and production of ova. It is commonly known that egg-laying in fowl may be stopped by various means without this being followed by broodiness. Again, we here find definitely that F.S.H. (+ unseparated thyreotropic) stops egg-laying without inducing broodiness, and that in this case ovulation ceases although the growth of ova continues (3, 4). It is certain therefore that mere blocking of egg-production and

ovulation does not necessarily result in the onset of broody behavior. It is nevertheless probably highly significant that, in association with its capacity to induce broodiness, prolactin (as now prepared) has also specific power to cause marked regression in the testes and ovaries of birds (3, 4, 10). Without this simultaneous action on the ovary the purpose served by broody behavior might be defeated in birds; while in mammals it is quite possible that prolactin is likewise the agent which represses ova formation and ovulation during pregnancy and lactation.

Since the incubation instinct (broody behavior) is clearly exhibited in some reptiles, amphibians and fishes the present demonstration of the rôle of prolactin in broodiness suggests that the phylogenetic origin of prolactin is to be sought in very low vertebrates or in still more ancient animals.

To the problem of behavior it is important that a known hormone has been identified as an essential element in an instinct—in this case the widespread variously modified and species-preserving incubation instinct. That instinct, with this clearly phyletic element not then identifiable, was well described by Whitman (11, 12). Wiesner and Sheard (13) have produced evidence that some substance or combination of substances arising in the anterior pituitary (and thought by them not to be the lactogenic factor) induces maternal behavior in the rat. Tietz (14) found that the involution of the corpus luteum resulting from injections of pregnancy urine in rabbits corresponds in time to the loosening of the ventral fur; she thinks an additional factor is necessary for excitation of the "nesting instinct" in does. Our results provide some probability that prolactin is that additional factor in the rabbit and rat.

SUMMARY

Prolactin has been identified as the hormone specifically concerned in the induction of broody behavior—the incubation instinct—in fowl. It is therefore presumably causally associated with the incubation instinct in much lower vertebrate animals, and quite possibly associated with maternal behavior in mammalian and human types.

A full expression of broodiness was obtained only in actively laying hens which are genetically capable of broodiness, and with dosage sufficient to repress the ovary. Partial broodiness, as expressed by clucking without nesting, is induced by prolactin in most laying and non-laying hens of a non-broody race, and in the non-laying hens of the so-called broody races. This partial response was also obtained in two mature roosters belonging to a race within which the hens may normally become broody.

Follicle-stimulating, thyreotropic (Schering-Kahlbaum), prolan (Elberfeld) and mare serum preparations are ineffective; effectiveness of a "luteinizing" pituitary principle is thus excluded. Prolactin heated to 100°C.

for 1 hour to destroy any growth principle is effective. Female sex hormone and corpus luteum hormone are ineffective.

This fourth response to prolactin is apparently closely associated with its previously known repressive effect upon the active mature gonads of birds, and with the crop-gland response in pigeons. Apparently all of these three responses in birds, like the known response (lactation) in mammals, are intimately associated with the care and feeding of the very young.

REFERENCES

(1) RIDDLE, O., R. W. BATES AND S. W. DYKSHORN. Proc. Soc. Exp. Biol. and Med. **29:** 1211, 1932.
(2) RIDDLE, O., R. W. BATES AND S. W. DYKSHORN. This Journal **105:** 191, 1933.
(3) BATES, R. W., E. L. LAHR AND O. RIDDLE. Anat. Rec. **57:** 30, 1933 (Supplement).
(4) BATES, R. W., E. L. LAHR AND O. RIDDLE. This Journal **111:** 361, 1935.
(5) WARREN, D. C. Personal communication.
(6) RIDDLE, O. Endocrinol. 1935 (Jan.-Feb.).
(7) WALKER, A. T. This Journal **74:** 249, 1925.
(8) NOETHER, P. Arch. f. Exp. Path. u. Pharmacol. **160:** 369, 1931.
(9) NOETHER, P. Klin. Wchnschr. **11:** 1702, 1932.
(10) RIDDLE, O. AND R. W. BATES. Endocrinol. **17:** 689, 1933.
(11) WHITMAN, C. O. Biological Lectures, Woods Hole, 285, 1898.
(12) WHITMAN, C. O. Posthumous Works, vol. 3, Carnegie Inst. of Wash., Publ. no. 257 (1919).
(13) WIESNER, B. P. AND N. M SHEARD. Maternal behavior in the rat. Oliver and Boyd, London, 1933.
(14) TIETZ, E. B. Science **78:** 316, 1933.

THE GROSS ACTION OF PROLACTIN AND FOLLICLE-STIMULATING HORMONE ON THE MATURE OVARY AND SEX ACCESSORIES OF FOWL[1]

ROBERT W. BATES, ERNEST L. LAHR AND OSCAR RIDDLE

From the Carnegie Institution, Station for Experimental Evolution, Cold Spring Harbor, N. Y.

Received for publication November 23, 1934

Other studies of this laboratory (1, 2) have shown that the testes of mature ring doves undergo rapid involution to less than one-tenth their former size during 10 to 12 days under heavy dosage with prolactin; and that follicle-stimulating hormone (F.S.H.) very markedly enlarges the mature testes and the mature ovaries of these doves. Opposed effects of these two hormones on testes and ovaries of mature pigeons have also been observed by us. In the present study, already reported in abstract (3), it was our purpose to learn whether prolactin and F.S.H. exercise opposite actions on the ovaries of mature fowl, and to make use of the large sex accessories (oviduct, comb, pubic bones) of these animals to learn whether and to what extent the output of ovarian hormone is influenced by these two pituitary principles.

In the case under study the gross effects of both hormones upon the weight and dimensions of the structures examined are so outstanding and informative that histological details are unnecessary in its initial description. Such details have been secured, however, on the smaller and more favorable structures obtainable from doves and pigeons.

MATERIAL AND METHODS. Since an accompanying paper (4) describes the breeds of fowl and the dosage used in the two studies the reader may consult that publication for information on those points. Prolactin and F.S.H. were prepared from the anterior pituitary essentially according to methods previously published (5). All fowl were killed on or soon after the last day (usually 7 to 20 days) of dosage. Though body weight differed somewhat in the various breeds, and in different lots of the same breed, and sometimes among individuals of the same lot, such differences do not markedly affect the values tabulated for groups of fowl. Trends of change in body weight during dosage are noted in the text.

RESULTS. *Effects of prolactin and F.S.H. on the ovary.* Ovaries of non-laying mature fowl (with previous record of egg-laying) are more

[1] Aided by a grant from the Carnegie Corporation of New York.

variable in weight than those of laying hens because such ovaries are small and contain variable quantities of detritus or "junk" retained in them during the involution accompanying temporary cessation of egg production. Some of this "junk"—dead and waste material—is present also in the ovaries of laying hens studied by us. An adequate measure of the amount of true ovarian material in a fowl requires the elimination of such detritus. Still another elimination must be made in weight of the "whole ovary" if one is to obtain accurate data on the amount of living and currently functioning ovarian tissue. In these ovaries a single ripe follicle may weigh more than 15 grams, of which about 95 per cent is—for present purposes—inert yolk; the other 5 per cent is actively functioning ovarian (follicular) tissue. For our "weights of ovary" we therefore found it necessary to obtain three sets of values: First, the "whole ovary" as dissected from the animal; second, the residue—after detritus and all follicles of more than 7 mm. had been removed; third, the amount of functioning follicular tissue present in the removed follicles. The two last-named values are combined, as "ovarian tissue" in our tabulated data. The weight of "total ovary" and the weight of living tissue thus derived each reveals an important aspect of the action of prolactin and F.S.H. on the fowl ovary.

Table 1 and figure 1 give results in summary form. The data fall into six groups of consistent contrasts, and demonstrate that *these two hormones act upon mature ovarian tissue in directly opposite* ways. Even the "resting" ovary—with its size and functions contracted by the mechanisms normally utilized by the body—is found to suffer a further reduction of one-fourth of its weight during 10 to 14 days of prolactin treatment.

An accompanying publication (4) gives some details concerning the number of days (usually 1 to 4) during which egg-laying may continue after beginning prolactin dosage, and also the time after last dosage (about 11 to 23 days) before egg-laying is resumed. Though F.S.H. stimulates the growth of ova it also stops egg-laying, usually after 1 to 3 days.

Effects of prolactin and F.S.H. on the sex accessories. Certainly oviducal size, possibly also comb size and width between pubic bones, reflects and measures the amount of estrin currently (or very recently) produced in the fowl ovary. Their fluctuations under dosage should provide additional tests of the capacity of prolactin to decrease and of F.S.H. to increase the amount of estrin-secreting tissue in the ovary. The data obtained, for all of these three characteristics in practically all of the six groups of tests, indicate that prolactin leads to a decrease and F.S.H. to an increase of the output of ovarian hormone. The striking and rapid reduction of oviducal (uterine) size as a secondary response to prolactin may prove to be a matter of much practical importance.

Effects of other hormones. Results obtained in one test with mare serum are wholly parallel to those obtained with F.S.H.; and, when doves are used as the test animal, mare serum seems to contain no anterior pituitary

TABLE 1

Opposite action of prolactin and follicle-stimulating hormone (F.S.H.) on the mature ovary of the fowl (all values are averages obtained for the various groups)

BREED OF FOWL	TREATMENT		NUMBER OF BIRDS	WEIGHTS OF OVARY		DIAMETER LARGEST NORMAL OVUM IN OVARY	WEIGHT OF OVIDUCT	CHANGE IN COMB DIMENSIONS	WIDTH OF PUBIC BONES, WITH CHANGE
	Duration	Hormone used (or control)		Whole ovary	Ovarian tissue			$\frac{H+L}{2}$	
			Non-laying fowl						
	days			*grams*	*grams*	*mm.*	*grams*	*mm.*	*mm.*
R. I. Reds		Control	7	5.5	3.0	5.8	7.0		30
	10	F.S.H.	12	25.3	5.3	18.0	36.8	+ 5	24 + 8
	11	Prolactin	12	4.0	2.3	3.9	4.5		25 − 0
White leghorns		Control	5	3.8	3.0	6.8	7.1		28
	16	F.S.H.	10	64.4	9.8	12.6*	28.4	+13	31 +10
	$17\frac{1}{2}$	Prolactin	10	2.7	2.6	3.7	3.7	− 5	31 − 2
Wyandottes		Control	2	4.8	4.8	4.9	22.0		37
	9	F.S.H.	5	74.8	11.6	24.6	42.0		32 +14
	10	Prolactin	5	4.4	2.9	5.5	5.1		32 − 2
			Laying fowl						
White Rocks		Control	2	37.0	7.9	31.5	45.0		48
	11	F.S.H.	5	117.4	22.7	24.9	64.6	+(?)	51 +3
	10	Prolactin	6	10.7	5.0	7.8	17.9	− 2	53 −11
White Leghorns		Control	2	48.0	9.0	32.0	45.0		43
	16	F.S.H.	1	187.0	(22.0)	28.3*	58.0	+24	50 + 5
	$13\frac{1}{2}$	Prolactin	6	6.2	4.3	8.8	12.7	− 7	46 −12
R. I. Reds		Control	3	46.0	8.7	31.7	58.3		42
	8	F.S.H.	3	123.7	13.4	28.5	53.0	+12	30 +18
	$5\frac{1}{2}$	Mare serum	1	122.0	11.9	34.5	87.0	+ 2	35 +10
	$6\frac{1}{2}$	Thyreotropic	3	56.7	7.7	33.4	50.2	+ 2	47 + 0
	10	Prolan (Elb.)	2	10.6	4.9	8.5	19.0	+ 0	42 − 7
	$5\frac{1}{2}$	Proluton	2	9.5	4.8	10.0	32.0	− 2	45 − 2
	11	Prolactin	3	5.5	3.7	5.5	13.9	− 0	38 − 7

* Under prolonged dosage the several largest ova become abnormal.

hormone other than F.S.H. The "thyreotropic" preparation used has been shown by us to contain very evident amounts of F.S.H.; the effects on ovary and sex accessories found here are doubtless ascribable to its

F.S.H. content. Prolan (Elberfeld) contains small amounts of F.S.H. as was proved by our tests on the immature dove testis, but also large amounts of "luteinizing" (and other) substance derived from pregnant urine. Two tests made with this combination of substances show that prolan has a strongly adverse effect upon active mature ovaries of fowl—a result in good agreement with that reported by this laboratory in 1931 (6) for immature ovaries of doves and pigeons.

The results of dosage with corpus luteum hormone (proluton) are particularly notable (4, for some details) because markedly adverse effects—much resembling those following prolactin—were found in a short term of dosage, and because the existence of a corpus luteum hormone (C.L.H.) in birds is a moot question. If a C.L.H. exists in the fowl the adverse effect

Fig. 1. Effect of prolactin and of F.S.H. dosage (both twice daily) on ovary and oviduct of laying fowl. *AA* is control. *93* was treated with prolactin, 25 units daily, for 10 days. *206* was treated with mare serum, 4 cc. daily for 5 days (killed at 8 days). *315*, not laying eggs, was treated with F.S.H., 4 mgm. daily for 18 days; ovary only shown.

of prolactin on the fowl ovary might be conceived as a two-phase action—the liberation of C.L.H. from the ovary (probably to be accomplished only by insult or degradation of normal ovarian tissue), and then a further anti-ovary action by this luteal hormone. This second phase of action might be a direct action upon the ovary, or an indirect one through the pituitary. Such a direct action would apparently involve the negation of the alleged rule or "principle" that an endocrine organ is not injured by an over-production of its own ineretion. Again, the mediation of C.L.H. can hardly be involved in the still more pronounced effect of prolactin on the bird testis.

We are much indebted to the Schering-Kahlbaum Co. and the Schering Corporation for the thyreotropic hormone and proluton used by us; to the Parke, Davis Research Laboratories for mare serum; and to the I. G. Farbengesellschaft, Elberfeld, Germany, and the Winthrop Chemical Co. for prolan.

Other effects of prolactin and F.S.H. on fowl. A slight tendency to loss of body weight in a majority of hens treated with prolactin is notable since most doves and pigeons similarly treated show a gain in weight. This loss in laying fowl (about 150 grams per bird) slightly exceeds the loss deriving from the involution of ovary and oviduct, and a loss occurred in nearly all hens; only two-thirds of the non-laying fowl lost weight—the average loss for all being about 100 grams, or less than 5 per cent of total body weight. These data, together with those obtained on doves (1, 2), show that the anti-gonad action of prolactin is not ascribable to an adverse effect on body weight.

Only about one-fifth of the hens treated with F.S.H. preparations showed a loss of body weight. The average gain for all, however, was probably not more than that represented by increased weight of ovary and oviduct. Over-filling the lower body cavity with ovary and ovarian eggs under F.S.H. stimulation sometimes compressed the pleural cavity and produced frank anoxemia. Hen 315, whose ovary of 285 grams (one-sixth of her total body weight) is shown in figure 1, was the most extreme case. This bird was a non-layer at the beginning of her 18 days of treatment, but when killed for measurements she was already nearly dead of asphyxia induced by pressure of the growing ovary upon her lungs.

In the fowl, as in doves and pigeons reported elsewhere (1, 2), the large ova produced under the stimulus of F.S.H. do not ovulate and form "eggs" in the usual manner. This of course partly accounts for the great weight of such "whole ovaries." In general, the follicles formed under this stimulus do not attain quite the full size at which they are normally extruded from the ovary. Instead, large numbers of follicles of sub-maximal size are formed, and some or many of these become "baggy"—with yolk deposited in numerous inter-follicular spaces. The ovary of one hen dosed only 9 days contained 76 follicles with a diameter exceeding 7 mm., and its largest normal follicle had a diameter of only 20 mm.

Elsewhere (4), it is shown that prolactin induces broodiness in laying fowl of broody races, and that other anterior pituitary hormones do not have a similar action. The data of table 1 show the nature and extent of change in ovary and sex accessories which accompanied that induction of broodiness by prolactin.

Discussion. Some investigators have indicated that crude or mixed anterior pituitary extracts gave adverse effects upon the gonad of one or another mammal, but the nature and identity of the adverse agent or agents were unknown. In 1926 Evans and Simpson (7) reported that the extract then used to produce gigantism in rats also "inhibited ovulation in females" and somewhat retarded testis development in males treated for months following the 21-day stage. Putnam, Benedict and Teel (8) later made comparable observations on the testes of dogs treated with a

similar pituitary preparation. This laboratory (9) has elsewhere noted the failure of both prolactin and F.S.H. to affect testis size in adult and old rats treated during 9 to 22 days. Otherwise than is noted above the mammalian ovary has yielded little from pituitary administration that investigators have interpreted as adverse or destructive action. Studies on that ovary, however, supply numerous accounts of phenomena of luteinization which, on the basis of more recent work, seem to be produced by a pituitary principle other than F.S.H. Satisfactory tests for contamination of our prolactin preparations with a luteinizing pituitary principle have not been completed; this laboratory, however, has repeatedly shown that the luteinizing principle obtained from pregnant urine is wholly unlike prolactin, and the known facts concerning the preparation of luteinizing factor from pituitary tissue suggest its presence not in prolactin but in the F.S.H. used in this study.

It would seem beyond question that the extensive and (often) cyclic size-increase of the oviducts of fowl and other birds is induced directly by estrin produced in the bird's ovary. Evidence that the pubic bones of birds normally relax or widen in response to the same hormone is inadequate, merely suggestive. The growth of the hen's comb while she passes from the non-laying to the laying condition is a commonplace observation of the barn-yard. It is highly probable that such comb-growth is induced by estrin. Measurements made in this study show that these three structures undergo change quite parallel to change in ovarian size. Growth of comb and oviduct in very immature chicks was observed by Domm and Van Dyke (10) to accompany early ovarian growth induced by the injection of an anterior pituitary extract (hebin) rich in F.S.H. Our measurements made on sex accessories of mature fowl, like those made on their ovaries, indicate that prolactin acts strongly and adversely on the germ gland.

The present study, like our earlier studies on the mature gonads of doves and pigeons, leads to the conclusion that the anterior pituitary elaborates two hormones which have opposite actions on mature gonads, both testis and ovary. The mechanism of prolactin's anti-gonad action is not definitely known. Reference has already been made to a conceivable (partial) mediation of corpus luteum hormone in some higher vertebrates. It is also conceivable that the injection of prolactin (and its normal release from the intact gland) effectively stops the output of F.S.H. from the pituitary, and that gonad atrophy thereafter proceeds as in the hypophysectomized animal.

When, where and to what extent in the vertebrate world the anti-gonad action of prolactin is utilized (in the two sexes) are questions little explored till now. Some light on these matters seems to be provided by the demonstration that broodiness in fowl is induced by prolactin (4). Broody (in-

cubation) behavior is essential to the propagation of the species, but the service of that behavior in fowl would be destroyed by an ovary that continued to produce eggs; it is here essential that broodiness be synchronized with an involuting gonad; prolactin promptly accomplishes both these things and their synchrony is thus assured. In the case of mammals it is quite possible that prolactin likewise represses ovulation and the later stages of follicular development during pregnancy and lactation. In mammals, however, corpus luteum hormone is certainly present; and if its action on the ovary from which it originates is similar to its action on two fowl ovaries observed by us, that hormone may largely share in repressing the mammalian ovary.

SUMMARY AND CONCLUSIONS

Data from 84 injected and 21 control fowl of three breeds show that prolactin and follicle-stimulating hormone (F.S.H.) promptly induce opposite effects upon the mature fowl ovary in both its laying and non-laying condition.

Prolactin dosage during 9 to 17 days decreased the true ovarian tissue of resting ovaries by 25 per cent, and that of active ovaries by 55 per cent. The weight of whole ovaries, including yolk, was decreased in laying birds to 10 or 20 per cent of their original weight.

Dosage with F.S.H. during 6 to 16 days increased the true ovarian tissue of resting ovaries by nearly 150 per cent, and that of active ovaries by 125 per cent. The weight of resting whole ovaries including yolk was increased approximately 1,000 per cent.

Oviducal or uterine size, certainly regulated by the estrin output of the ovary, is decreased by prolactin. For three groups of non-laying fowl decreases of 36, 48, and 91 per cent, and for three groups of laying fowl decreases of 60, 72 and 74 per cent, were found. Possible medical applications of this secondary action of prolactin—reduction of uterine size and inhibition of uterine growth—are apparent but largely unexplored.

Weight of oviduct was increased by F.S.H. in three groups of non-laying fowl by 425, 300 and 90 per cent, and in three groups of layers by 44, 29 and 5 per cent.

Size of comb and space between pubic bones decreased under prolactin dosage and increased under F.S.H.

Indirectly prolactin decreased and F.S.H. increased the output of estrin by the fowl ovary.

Prolan and corpus luteum hormone in a total of only four tests gave definitely adverse effects on active mature ovaries.

Data concerning the nature and extent of the ovarian changes which accompany the induction of broody behavior by prolactin are presented.

REFERENCES

(1) RIDDLE, O. AND R W. BATES. Endocrinol. **17:** 689, 1933.
(2) RIDDLE, O., R. W. BATES AND E. L. LAHR. Proc. Soc. Exp. Biol. and Med. (in press).
(3) BATES, R. W., E. L. LAHR AND O. RIDDLE. Anat. Rec. **57:** no. 4 Supplement, 30, 1933.
(4) RIDDLE, O., R. W. BATES AND E. L. LAHR. This Journal **111:** 352, 1935.
(5) RIDDLE, O., R. W. BATES AND S. W. DYKSHORN. This Journal **105:** 191, 1933.
(6) RIDDLE, O. AND I. POLHEMUS. This Journal **98:** 121, 1931.
(7) EVANS, H. M. AND M. E. SIMPSON. Anat. Rec. **32:** 206, 1926.
(8) PUTNAM, T. J., E. B. BENEDICT AND H. M. TEEL. Arch. Surg. **18:** 774, 1929.
(9) RIDDLE, O., E. L. LAHR, R. W. BATES AND C. S. MORAN. Proc. Soc. Exp. Biol. and Med. **32:** 509, 1934.
(10) DOMM, L. V. AND H. B. VAN DYKE. Proc. Soc. Exp. Biol. and Med. **30:** 351, 1932.

SOME METABOLIC EFFECTS OF CLAMPING VISCERAL ARTERIES, SPLANCHNIC VASOCONSTRICTION AND ADRENAL AND HEPATIC STIMULATION; WITH SPECIAL REFERENCE TO THE CALORIGENIC ACTION OF ADRENIN AND SYMPATHIN

FRED R. GRIFFITH, JR. AND F. E. EMERY

From the Department of Physiology, University of Buffalo, Buffalo, N. Y.

Received for publication August 3, 1934

This work was the outgrowth of curiosity as to the effect of stimulating the hepatic nerves on metabolic rate and blood lactic acid; this, in turn, is related to the larger problem of the rôle of the liver in the calorigenic response to adrenalin; we were led, therefore, to evaluation of this for the conditions of our experiments, an increased discharge of adrenin being secured by splanchnic stimulation. Since stimulation of either splanchnic or hepatic nerves produces, among their other effects, intense vaso-constriction and diminished blood supply in their respective vascular beds, the result of this alone was determined 1, by stimulation of the splanchnic after removal of the adrenal and cutting the nerves to the liver, as well as 2, by merely clamping the arteries to the viscera.

METHODS. Cats anesthetised with chloralose were used throughout. Their rectal temperature was kept normal and as constant as possible by an electrically heated animal holder. Expired air was obtained from the tracheal cannula and metered in a small, specially designed spirometer of about 3 liters' capacity. The outdoor, inspired air was separated from that expired by water valves having a dead-space approximately equal to that of the natural, upper respiratory passages. Between the expiratory valve and the spirometer a by-pass permitted withdrawal over mercury of a sample for analysis into a calibrated sampling tube which filled slowly during the period of collection; this varied with the size and condition of the animal, being usually from 3 to 5 minutes and always the time required to fill the spirometer and sampling tube between marks.

Blood pressure was taken from one carotid artery; a cannula in the other carotid permitted withdrawal of blood samples (2 cc.) for determination of sugar and lactic acid; the former by the method of Hagedorn and Jensen, the latter by that of Friedemann, Cotonio and Shaffer, and both on the same Folin-Wu tungstic acid filtrate.

The abdominal viscera were reached through a ventral mid-line incision;

further preparation differed according to the contemplated experimental procedure.

1. Stimulation of hepatic nerves. These were secured where they accompany the hepatic artery central to the gastro-duodenal branch (Cannon and Uridil, 1921), ligated, cut centrally and placed on shielded electrodes. Most of the fibers so obtained terminate in the liver; those distributed with the gastro-duodenal artery were cut in two experiments without effect. Often both adrenals were removed to preclude possibility of involvement in the response.

2. Stimulation of left splanchnic-adrenal. The left splanchnic, cut centrally, was placed on shielded electrodes; preliminary to this the hepatic nerves were cut as above to prevent excitation from reaching the liver; usually, also, the right adrenal was removed. Stimulation would affect the splanchnic vascular bed (exclusive of liver) and the left adrenal.

3. Stimulation of left splanchnic. Prepared as in 2, plus removal of left adrenal; stimulation affects only the splanchnic vascular bed, exclusive of the liver.

Frequently electrodes were placed on both hepatic and splanchnic nerves during the preparation so either could be stimulated in turn without delay or further disturbance of the animal (see figure).

Excitation was by tetanizing current from a Harvard inductorium; it was not continuous but by means of an automatic interrupter in the primary was on and off alternately for 5-second intervals; the total period of stimulation varied from 10 to 20, but usually was about 15 minutes.

4. Clamping visceral arteries. The coeliac distal to its hepatic branch and the superior and inferior mesenterics were freed for a short distance of connective tissue and plexus and provided with loose ligatures so as to be quickly accessible when needed. The approach being from the left side, the left adrenal was removed to prevent stimulation of it when the clamps were applied; they were left on 5 minutes.

After completion of the operative procedures the tracheal cannula was connected with the respiratory valves and 30 to 45 minutes allowed for subsidence of metabolic disturbances and attainment of equilibrium under the new breathing conditions against the slight pressure and possibly abnormal dead-space of the valves. Two collections of expired air and blood, 15 to 20 minutes apart, were then secured as normals; following the second, registration of blood pressure was begun and continued throughout the experiment.

Within 30 to 60 seconds after beginning stimulation or application of the clamps, collection of expired air would be under way; this collection, requiring 3 to 5 minutes, depending on the size and condition of the cat, would be followed immediately by another; subsequent samples at intervals, first of 5 and later 10 minutes, covered the remainder of the period of

stimulation and recovery (see graph). Blood samples were taken at approximately the middle of the periods of air collection.

RESULTS. The effects of stimulating 1, hepatic nerves; 2, splanchnic-adrenal; and 3, splanchnic alone, are shown in table 1; of 4, clamping visceral arteries, in table 2; typical responses to 1 and 2 are also illustrated in the graph. Our interest centers chiefly in the changes of blood lactic acid and oxygen consumption; blood pressure and heart rate, pulmonary ventilation, respiratory quotient and blood sugar are of incidental importance in evaluating the total metabolic response to each procedure and will be referred to as occasion demands for this purpose.

Preliminary hepatic and splanchnic-adrenal stimulation disclosed invariable increase of blood lactic acid; oxygen intake usually rose but occasionally fell. This seemed to indicate the operation of two opposing factors; *a*, vasoconstriction with asphyxial accumulation of lactic acid and diminished oxygen intake; *b*, liberation (usually) in increased amounts of adrenin, or, from the liver, an adrenin-like substance (sympathin) increasing oxygen consumption. Effort to disentangle these opposing influences led to the later experiments, designed to demonstrate the uncomplicated effects of visceral anemia and anoxemia; logical exposition favors their consideration first.

Clamping visceral arteries cuts off the oxygen supply to the viscera involved, entailing diminished oxygen consumption of the animal as a whole (−12 per cent; 0.6 cc./kg./min.; table 2, III). The local anoxemia leads to asphyxial accumulation of lactic acid which does not reach its maximum in the general circulation until blood flow through the occluded areas is restored and flushes them out (+79 per cent; 6 mgm. per cent; table 2, VI); this elevates respiratory quotient and pulmonary ventilation (table 2, IV and V); blood sugar increases only slightly and temporarily (table 2, VII).

The chief significance of these results for our purpose is the measure they provide of the decrease in oxygen consumption and increase of blood lactic acid that may be expected from visceral anemia; this is not to imply that such changes, in the other experiments, can arise only in this way; but to the extent this factor is involved and the results are within these limits its operation may be strongly suspected.

Important, also, though blood lactic acid remained high, oxygen intake returned only to its previous normal level; exemplifying a lack of correlation characteristic of all that follows.

Splanchnic vasoconstriction produced very similar effects; oxygen consumption fell not quite so much (− 6 per cent; 0.4 cc./kg./min.; table 1, III, 3); but lactic acid rose precisely 6 mgm. per cent (+ 51 per cent; table 1, VI, 3) as before. Pulmonary ventilation presents the only qualitative difference (table 1, V, 3), decreasing instead of increasing; this is of

TABLE 1

Averages of experiments involving stimulation of (I) hepatic nerves; (II) left splanchnic-adrenal (hepatic nerves cut); and (III) the left splanchnic alone (adrenal out and hepatic nerves cut)

Stimulation rarely less than 10 or more than 20 and usually for 15 minutes.

Due to lack of precise synchronism between individual periods of collection or sampling, these averages, though useful for quantitative comparison, involve some distortion of the time relations; compare the data for increased oxygen consumption with those illustrated in the figure.

PROCEDURE	NO. OF EXPS.	NORMAL	PER CENT OF NORMAL AND MINUTES AFTER BEGINNING STIMULATION							
			0–1	2–3	4–5	6–7	8–10	11–15	16–20	21–25
I. Blood pressure										
1. Stimulate hepatic nerves										
a. Oxygen increased	11	132	147	149	141	110	110	94	72	
b. Oxygen decreased	8	125	149	147	125	97	85	98	94	
2. Stimulate splanchnic-adrenal										
a. Oxygen increased	5	99	229	207	169	103	100	109		
b. Oxygen decreased	5	86	276	206	232	164	123	135		
3. Stimulate splanchnic	10	98	160	165	136	108	105	96	97	
II. Heart rate per minute										
1. Stimulate hepatic nerves										
a. Oxygen increased	11	199	94	96	97	100	98	98	100	
b. Oxygen decreased	8	194	92	92	93	95	91	100	100	
2. Stimulate splanchnic-adrenal										
a. Oxygen increased	5	200	112	94	100	98	99	99	97	
b. Oxygen decreased	5	234	80	78	87	88	91	97		
3. Stimulate splanchnic	10	214	88	94	91	96	94	100	97	
			0–3		4–6	7–10	11–15	16–20	21–25	
III. Oxygen consumption (cc./kg./min.)										
1. Stimulate hepatic nerves										
a. Oxygen increased	26	6.2	109		106	105	105	105	102	
b. Oxygen decreased	8	6.4	93		93	96	96	98		
2. Stimulate splanchnic-adrenal										
a. Oxygen increased	13	6.0	110		107	108	107		97	
b. Oxygen decreased	8	6.0	94		96	99		102		
3. Stimulate splanchnic	10	5.8	94		94	94	94	98	100	
IV. Respiratory quotient										
1. Stimulate hepatic nerves										
a. Oxygen increased	26	0.74	103		104	101	101	99		
b. Oxygen decreased	8	0.71	107		107	104	104	104		
2. Stimulate splanchnic-adrenal										
a. Oxygen increased	13	0.74	107		111	111	107	105	105	
b. Oxygen decreased	8	0.78	99		104	104	109			
3. Stimulate splanchnic	10	0.74	103		103	103	103	107		

TABLE 1—*Concluded*

PROCEDURE	NO. OF EXPS.	NORMAL	PER CENT OF NORMAL AND MINUTES AFTER BEGINNING STIMULATION					
			0–3	4–6	7–10	11–15	16–20	21–25
V. Pulmonary ventilation (cc./min.)								
1. Stimulate hepatic nerves								
a. Oxygen increased	26	358	104	103	103	104	103	100
b. Oxygen decreased	8	305	101	101	101	106	115	
2. Stimulate splanchnic-adrenal								
a. Oxygen increased	13	356	106	106	109	104		
b. Oxygen decreased	8	383	93	92	101	109		
3. Stimulate splanchnic	10	320	96	96	98	99		
			0–5	6–10	11–15	16–20	21–25	
VI. Blood lactic acid (mgm. per cent)								
1. Stimulate hepatic nerves								
a. Oxygen increased	15	11.9	116	138	124			
b. Oxygen decreased	8	9.4	113	131		145		
2. Stimulate splanchnic-adrenal								
a. Oxygen increased	6	15.6	133	145		131		
b. Oxygen decreased	4	18.4	110	123		121		
3. Stimulate splanchnic	10	11.5	128	137	151	152		
VII. Blood sugar (mgm. per cent)								
1. Stimulate hepatic nerves								
a. Oxygen increased	16	161	123	137	142	129		
b. Oxygen decreased	8	174	124	129	125	126		
2. Stimulate splanchnic-adrenal								
a. Oxygen increased	6	131	111	112	151		136	
b. Oxygen decreased	4	148	114	120	116		112	
3. Stimulate splanchnic	10	162	99	103	103	106	108	

TABLE 2

The effect of clamping the visceral arteries for five minutes; averages of 9 experiments

PROCEDURE	NORMAL	PER CENT OF NORMAL			
		Clamps on	Time after removal of clamps (min.)		
			0–5	10–15	25–30
I. Blood pressure	125	121	96	80	
II. Heart rate per minute	219	98	102	99	
III. Oxygen consumption (cc./kg./min.)	5.3	88	103	104	95
IV. Respiratory quotient	0.75	111	112	104	111
V. Pulmonary ventilation (cc./min.)	264	103	118	126	121
VI. Blood lactic acid (mgm. per cent)	7.4	148	179	158	161
VII. Blood sugar (mgm. per cent)	152	99	118	110	101

no importance in itself but merely as a characteristic uniting these and the following experiments, in which it appears to follow less the blood lactic acid than metabolic rate.

When the adrenal is intact during splanchnic stimulation there are two possibilities: 1, if, due to depth of anesthesia, previous exhaustion of adrenin through stimulation incident to the operative preparation (McIver and Bright), or other causes, its secretion is not significantly increased, results might be expected similar to those just described. This was true in 8 of the 21 experiments of this kind; in these the decrease in oxygen consumption (−6 per cent; 0.4 cc./kg./min.) and pulmonary ventilation (table 1, III and V, 2b) and increase of blood lactic acid (+23 per cent; 4.2 mgm. per cent) and respiratory quotient (table 1, VI and IV, 2b) are of the same order as those resulting from splanchnic stimulation alone. Three differences: greater increase of *a*, blood sugar (table 1, VII, 2b vs., 3) and *b*, blood pressure (table 1, I, 2b vs., 3); and *c*, beginning of return toward normal of the depressed oxygen consumption even while stimulation (and vasoconstriction) is in progress, indicate adrenin output probably was increased somewhat, though not enough completely to counteract the metabolic-depressive effect of the accompanying vasoconstriction. And though not comparable in all respects (as increased blood pressure), due perhaps to difference of species (see Cori, Cori and Buchwald, 1930, for rabbit) and experimental conditions, this recalls the finding of Cori and Buchwald (1930) that minimal effective doses of adrenalin, intravenously in man, increase blood sugar although a doubled rate of injection is required to affect blood lactic acid and metabolic rate.

2. If splanchnic-adrenal stimulation is successful in significantly increasing the output of adrenin, the tendencies just noted should be intensified and especially its calorigenic action might be expected completely to overcome the depressed oxygen consumption due to accompanying visceral anemia. This apparently was realized in 13 of the 21 experiments of this kind in which oxygen intake increased 10 per cent (table 1, III, 2a). Corroborative evidence of a more effectively increased output of adrenin may be seen in *a*, the greater increase of blood sugar (table 1, VII, 2a) and *b*, an increase rather than decrease of heart rate (table 1, II, 2a vs., 2b); indicating adrenin in sufficient amount to overcome the depressor reflex. Something also might be made of the greater increase of blood lactic acid occurring here (45 per cent; 7 mgm. per cent; table 1, VI, 2a) especially in light of the experience of Cori and Buchwald cited above. In view of the large individual variations, however, and the small number of determinations in group 1 (table 1, VI, 2b), equal, if not more weight should be given to the increase, greater in percentage (51) and practically the same in actual amount (6 mgm. per cent) produced by splanchnic constriction alone (table 1, VI, 3); indicating that under the conditions of these experi-

ments the calorigenic action of adrenin was unaccompanied by any significant addition to the lactic acid of the blood.

Stimulation of the hepatic nerves produced results so similar to those just described as to require little additional comment. Of a total of 34 experiments, oxygen consumption decreased in 8 and increased in 26 (table 1, III, 1a and 1b). Irrespective of this difference, the other effects are quite alike (see footnote) and, except for the hyperglycemia which is an expected specific result of hepatic glycogenolysis, are similar to those we have learned to recognize as attributable to vasoconstriction. Hepatic nerve stimulation, causing vasoconstriction in the liver (Griffith and Emery, 1930) like splanchnic constriction or clamping the visceral arteries, increases blood lactic acid (table 1, VI, 1a and 1b) (Macleod and Wedd, 1914) and diminishes oxygen intake. That the oxygen consumption, on the other hand, usually increases, indicates the operation in such instances of some countervailing influence on the metabolism. Whether this is success or failure (according to the condition of the animal) of excitation to augment some intrahepatic activity or the output of an adrenin-like hormone (sympathin; Cannon and co-workers, 1921–1933) having similar calorigenic action, cannot be decided from these data. The latter seems more reasonable; for it is difficult to believe the metabolism of the liver could be increased enough to elevate general oxygen consumption even the average 9 per cent and much less the 20 to 30 per cent occasionally observed; and it is not unreasonable to postulate this additional adrenin-like property for hepatic sympathin.

As has been said, except as regards oxygen consumption, the results here are substantially alike; i.e., there is no correlation between this variable and the changes in blood sugar, lactic acid, pressure or heart rate (compare 1a and 1b, I–VII, table 1); that pulmonary ventilation and respiratory quotient show no significant differences paralleling the changes of metabolic rate, is the only serious failure among these data to observe expected physiological relationships.

The alterations in metabolic rate are transient. It is impossible to keep the metabolic rate permanently elevated by either splanchnic-adrenal or hepatic nerve stimulation. This is evident in the averages of table 1 (III, 1a and 2a) but more conspicuously in the individual experiments of which two are shown in the graph. Maximum oxygen consumption occurs during the first five minutes or so and then begins to return to normal which it may reach or even fall below in spite of continued stimulation.

It seems unlikely that this can be due to fatigue at the electrodes, especially when it is recalled that the stimulation was interrupted every 5 seconds by an equal period of rest; nor can it be due to injury at the electrodes, since, without changing their position on the nerve, the effect can be repeated after a sufficient rest period. Thus in one instance initial

stimulation of the hepatic nerves increased oxygen intake 28 per cent; 70 minutes later the same stimulation again caused an increase of 16 per cent. Splanchnic-adrenal stimulation which caused an increase of 11 per cent, 120 minutes later produced a rise of 36 per cent. From this it would seem that subsidence of the metabolic effect during any one period of stimulation is due to fatigue or exhaustion of the end organs involved, the liver or adrenal. No previous observations of this kind regarding the output of adrenin are known to us, though similar evidence is at hand concerning the cardio-accelerator substance from the liver (Cannon and Griffith, 1922) and sympathin from other sources (Rosenblueth and Schlossberg, 1931).

Blood pressure behaves similarly (table 1, I); this, however, does not

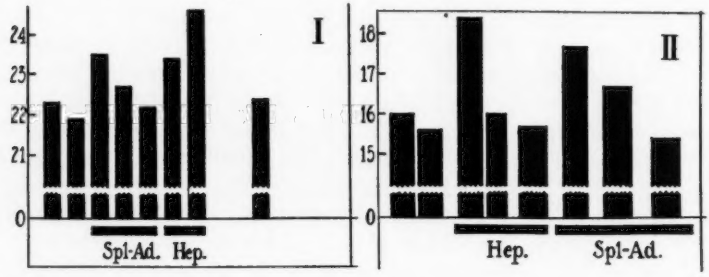


Fig. 1. I. Experiment 2-2-31. Normal oxygen consumption per minute: 12:10–14, 22.3 cc.; 12:16–20, 21.9 cc. Stimulate left splanchnic and adrenal, 12:22–38; oxygen consumption per minute: 12:22–25, 23.5 cc.; 12:26–29, 22.7 cc.; 12:36–39, 22.2 cc. Stimulate hepatic nerves, 12:39–49; oxygen consumption per minute: 12:40–43, 23.4 cc.; 12:44–47, 24.6 cc.; 1:02–06, 22.4 cc.

II. Experiment 4-21-31. Normal oxygen consumption per minute: 12:33–39, 16.0 cc.; 12:40–46, 15.6 cc. Stimulate hepatic nerves, 12:49–1:13; oxygen consumption per minute: 12:50–56, 18.4 cc.; 12:57–1:02, 16.0 cc.; 1:05–12, 15.7 cc. Stimulate left splanchnic and adrenal, 1:14–48; oxygen consumption per minute: 1:16–22, 17.7 cc.; 1:26–33, 16.7 cc.; 1:38–45, 15.4 cc.

imply fatigue of the constrictor mechanism. Table 2 gives the average increase in blood pressure during the entire 5 minutes clamps were applied to the visceral arteries; more detailed analysis of this period shows that immediately upon application of the clamps the pressure rises an average of 36 per cent and then immediately begins to fall until at the end of the 5 minutes and while the clamps are still in place it is only 8 per cent above normal. Failure of the blood pressure to remain elevated must in this case and presumably also when the nerves are stimulated, be due to dilatation in some part of the periphery. This parallelism between blood pressure and oxygen consumption in those instances in which the latter was increased, as noted previously by Hunt and Bright (1926), implies no causal

relationship; for the same increases of pressure occurred even though oxygen intake fell.

Finally, it may be noted that elevation of blood sugar and lactic acid is not thus transient; a causal relationship with oxygen consumption even when this increases would therefore seem precluded.

Comparison of response to hepatic and adrenal stimulation. McIver and Bright (1924), in the course of a careful study of the effect of splanchnic-adrenal stimulation on gaseous metabolism, reported a few incidental observations made after removal of the adrenal but with the hepatic nerves intact, from which they concluded the hepatic effect was uncertain and small compared with that of the adrenal. In our own work, out of 35 experiments, stimulation of the hepatic nerves produced no change in oxygen consumption in one; in 8 there was a decrease varying from −2 to −16, average, −7 per cent; and in 26 the rate was increased +3 to +28, average, +9 per cent. Considering only these positive responses, the average is indeed still below the average increase of 20 per cent obtained by McIver and Bright from adrenal stimulation. They emphasize, however, that the magnitude of the adrenal response depends on experimental conditions, of which anesthesia and the condition of the glands resulting therefrom received special consideration by them. It was for the purpose of making a comparison valid for our conditions that we repeated their work on splanchnic-adrenal stimulation. Our results confirm theirs that this also may cause either decrease or increase of metabolic rate; the increases under the anesthesia and operative technique used by us ranged from +4 to +36, average, +10 per cent; a range and average practically the same as we obtained from stimulation of the liver.

The rôle of the liver in the calorigenic response to adrenin. The evidence at present available is about equally divided as to whether the liver is (Crile and Rowland, 1922; Caskey and Humel, 1926; Caskey, 1927; Soskin, 1927) or is not (Hunt and Bright, 1926; Cori and Buchwald, 1931) essential for the calorigenic response to adrenalin. Since our results, just cited, show that stimulation of the liver through its nerves produces, on the average, the same increase of metabolic rate as adrenin discharged by splanchnic stimulation (table 1, III, 1a and 2a) the question arises as to whether the hepatic effect accounts for the adrenin response; i.e., does adrenin going to the liver act on terminals of the same nerves we stimulate artificially and so produce its calorigenic effect? We believe not. If, as noticed above, the liver is "fatigued" by stimulation of its nerves, an immediate discharge of adrenin, by stimulation of the splanchnic, produces a typical response (fig. II). Conversely, if this is due to the action of this hormone on the nerve endings in the liver, its subsidence ought to indicate the same degree of hepatic exhaustion as results from direct stimulation of the liver nerves; yet this, after subsidence of the adrenin effect, produces an

immediate, successive elevation of metabolic rate (fig. I). Averages of those experiments for which we have double stimulation show that initial excitation of hepatic nerves increases oxygen consumption 10 per cent, and immediately after splanchnic-adrenal response, 11 per cent; also, initial splanchnic-adrenal stimulation causes an average increase of 14 per cent, and immediately after the liver response, 15 per cent. We conclude, therefore, that the liver is not involved in the calorigenic response to adrenin.

The cause of the increased metabolic rate occurring in our experiments. As has been shown in the preceding sections there is no parallelism between the changes in oxygen consumption and 1, work of the heart; 2, carbohydrate plethora; or 3, blood lactic acid concentration; the first is confirmation of McIver and Bright (1924) and Hunt and Bright (1926), and the second of Boothby and Sandiford (1923) for the calorigenic action of adrenalin; the third is significant in two respects: 1, under the conditions of these experiments, increased blood lactic acid, *per se*, did not elevate metabolic rate; this is not to say that under other conditions it may not (Abramson, et al., 1927; Dietrich and Zehen, 1932; Buchwald, Cori and Fischer, 1933), but merely confirms previous experience as to the ease with which this effect is suppressed (see Long and Horsfall, 1932), or failure of the two to vary proportionately, as after: *A*, exercise (Martin, et al., 1929; Margaria, et al., 1933); *B*, administration of pitressin (Himwich and Fazikas, 1930; Himwich and Haynes, 1931); and *C*, adrenalin, itself, *a*, in man (Cori and Buchwald, 1930) and *b*, in hepatectomized frogs (Cori and Buchwald, 1931; Buchwald and Cori, 1931).

2. The calorigenic action of adrenin or hepatic sympathin was therefore independent of and something additional to any possible effect of increased blood lactic acid; this is not to question (Wiggers, 1934, p. 1037) that it is an expression of increased lactic acid metabolism (Cori, 1931, p. 240); it merely means that if adrenalin mobilizes muscle glycogen (Cori and Cori, 1928; Major and Mann, 1932), lactic acid so formed stimulates oxidation more effectively than that supplied by the blood. Also, since increased metabolic rate in these experiments was accompanied by no significant increase in blood lactic acid above that accountable for by visceral anemia, it follows that in amounts necessary to increase oxygen consumption at least 10 per cent, the cycle is completed within the cells with little or no escape into the circulation. This is not surprising since with much greater increases in metabolic rate, as after protein feeding and in hyperthyroidism (Adams and Foster, 1928) or exercise up to two-thirds of the maximal rate of work (Owles, 1930; Cook and Hurst, 1933; Margaria, Edwards and Dill, 1933) the concentration of lactic acid in the blood is not raised.

Is increased blood lactic acid accompanying the calorigenic action of

adrenalin due, then, to vasconstriction (Macleod, 1929)? We believe it is entirely a matter of dosage, but, particularly, that within physiological limits, argument from blood pressure changes (Cori, Cori and Buchwald, 1930) may be quite misleading. It has already been mentioned that even during the time clamps were on the visceral arteries, so there could be no question of the effective occlusion of the splanchnic circulation, blood pressure returned practically to normal; this also occurred during splanchnic and splanchnic-adrenal stimulation (table 1, I, 2a, 2b and 3) and must have meant an actual improvement of the peripheral circulation; which is exactly the sort of preferential effect accomplished by physiological doses of adrenalin (Hartman, et al., 1915–1929; Hoskins, et al., 1916; Clark, 1930–1934). From which it is evident adrenalin could maintain a prolonged visceral constriction causing increased blood lactic acid, without alteration of general blood pressure and with actual improvement of muscle circulation at a time when it is most needed. With larger doses, peripheral constriction in conjunction with the specifically augmented glycogenolysis would add to the blood lactic acid.[1]

SUMMARY

This report describes metabolic effects produced by 1, clamping the visceral arteries; 2, stimulation of the splanchnic (adrenal out and hepatic nerves cut); 3, splanchnic-adrenal stimulation (hepatic nerves cut), and 4, stimulation of the nerves to the liver.

All of these increase blood lactic acid in comparable amount; decreased oxygen consumption resulted invariably from 1 and 2 and occasionally from 3 and 4; these are attributed to visceral anemia.

Increased oxygen consumption resulted usually from 3 and 4. This is ascribed to adrenin or, from the liver, adrenin-like sympathin, produced usually in amounts sufficient to overcome the metabolic-depressive effect

[1] To complete the record mention must be made of five experiments in which stimulation of the hepatic nerves was accompanied by decrease in blood lactic acid to 81, 91 and 93 per cent, at the 5, 10 and 15 minute intervals, respectively. The possibility of accelerating the synthesis of blood lactic acid to carbohydrate which is now a well established liver function (Cori and Cori, 1929; Himwich, Koskoff and Nahum, 1929–30; Nitzescu and Monteanu, 1931; Grant, 1933), by stimulation of the hepatic nerves, is not entirely unreasonable and might even be expected as the logical compliment of the glycogenolytic reaction which they are known to facilitate. Nevertheless, although we know of no technical fault in these determinations, though nothing of the kind was observed in connection with any of the other procedures, and though such a reaction might be expected to be obscured by the concomitant lactic acidemia of vasoconstrictor origin, this result was too infrequent to be taken seriously at present. In these experiments the oxygen consumption was increased and in spite of the decreased blood lactic acid, and as further evidence of genuine acceleration of metabolic rate, pulmonary ventilation and respiratory quotient were also augmented.

of the accompanying vasoconstriction. Whether resulting from adrenal or hepatic stimulation, these increases were very alike in magnitude and time relations. Nevertheless we believe them independent reactions and, particularly, that the calorigenic action of adrenin is not merely an expression of this possible hepatic response.

Considered together, these results are significant as indicating that under the conditions of these experiments (anesthesia, etc.) increases of blood lactic acid, *per se*, had no effect on metabolic rate, whereas adrenin and hepatic sympathin did; and if this calorigenic action is ascribable to increased lactic acid metabolism, this must have been confined to the tissue (muscle) cells and was unaffected by and contributed nothing to the lactic acid of the blood.

Incidental consideration is given to changes in blood sugar, blood pressure, heart rate, pulmonary ventilation and respiratory quotient.

REFERENCES

ABRAMSON, H. A., M. G. EGGLETON AND P. EGGLETON. J. Biol. Chem. **75:** 763, 1927.
ADAMS, L. E. AND G. L. FOSTER. Proc. Soc. Exp. Biol. Med. **25:** 310, 1928.
BOOTHBY, W. M. AND I. SANDIFORD. This Journal **66:** 93, 1925.
BUCHWALD, K. W. AND C. F. CORI. J. Biol. Chem. **92:** 355, 1931.
BUCHWALD, K. W., C. F. CORI AND R. E. FISHER. Ibid. **103:** 763, 1933.
CANNON, W. B. AND D. RAPPORT. This Journal **58:** 308, 1921.
CANNON, W. B. AND J. E. URIDIL. Ibid. **58:** 353, 1921.
CANNON, W. B. AND F. R. GRIFFITH. Ibid. **60:** 544, 1922.
CANNON, W. B. AND Z. M. BACQ. Ibid. **96:** 392, 1931.
CANNON, W. B. AND A. ROSENBLUETH. Ibid. **104:** 557, 1933.
CASKEY, M. W. Ibid., **80:** 381, 1927.
CASKEY, M. W. AND E. J. HUMEL. Ibid. **76:** 213, 1926.
CLARK, G. A. J. Physiol. **69:** 171, 1930; **80:** 429, 1934.
COOK, L. C. AND R. H. HURST. Ibid. **79:** 443, 1933.
CORI, C. F. Physiol. Reviews **11:** 205, 1931.
CORI, C. F. AND K. W. BUCHWALD. This Journal **95:** 71, 1930.
J. Biol. Chem. **92:** 367, 1931.
CORI, C. F. AND G. T. CORI. Ibid. **79:** 309, 321, 1928.
Ibid. **81:** 389, 1929.
CORI, C. F., G. T. CORI AND K. W. BUCHWALD. This Journal **93:** 273, 1930.
CRILE, G. W. AND A. F. ROWLAND. Ibid. **62:** 370, 1922.
DIETRICH, S. AND M. ZEHEN. Ztschr. klin. Med. **120:** 517, 1932.
GRANT, R. J. Physiol. **80:** 41, 1933.
GRIFFITH, F. R., JR. AND F. E. EMERY. This Journal **95:** 20, 1930.
HARTMAN, F. A. Ibid. **38:** 438, 1915.
HARTMAN, F. A. AND MCPHEDRAN. Ibid. **43:** 311, 1917.
HARTMAN, F. A., J. I. EVANS AND H. G. WALKER. Ibid. **85:** 91, 1928; **90:** 668, 1929.
HIMWICH, H. E. AND J. FAZIKAS. Proc. Soc. Exp. Biol. Med. **28:** 331, 1930.
HIMWICH, H. E. AND F. W. HAYNES. This Journal **96:** 640, 1931.
HIMWICH, H. E., Y. D. KOSKOFF AND L. H. NAHUM. J. Biol. Chem. **85:** 571, 1929–30.
HOSKINS, R. G., R. E. L. GUNNING AND E. L. BERRY. This Journal **41:** 513, 1916.
HUNT, H. B. AND E. M. BRIGHT. Ibid. **77:** 353, 1926.

McIver, M. A. and E. M. Bright. Ibid. **68**: 622, 1924.
Macleod, J. J. R. Lancet **2**: 1, 55, 1929.
Macleod, J. J. R. and A. M. Wedd. J. Biol. Chem. **18**: 447, 1914.
Major, S. G. and F. C. Mann. This Journal **101**: 462, 1932.
Margaria, R. and H. T. Edwards. This Journal **107**: 681, 1931.
Margaria, R., H. T. Edwards and D. B. Dill. Ibid. **106**: 689, 1933.
Martin, E. G., J. Field and V. E. Hall. Ibid. **88**: 407, 1929.
Nitzescu, I. I. and N. Munteanu. C. R. Soc. Biol. Paris **108**: 294, 1931.
Owles, W. H. J. Physiol. **69**: 214, 1930.
Rosenblueth, A. and T. Schlossberg. This Journal **97**: 370, 1931.
Soskin, S. Ibid. **83**: 162, 1927.
Tangl, E. Pflüger's Arch. **6**: 563, 1895 (from Schmidt's Jahrb. f. ges. Med., **250**: 6, 1896.
Wiggers, C. J. Physiology in health and disease. Philadelphia, 1934.

CHARACTERISTIC VARIATIONS IN COMBINATIONS OF LINEAR CHEST ELECTRODES (MULTI-PLANE CHEST LEADS) RESULTING FROM EXPERIMENTAL VENTRICULAR LESIONS

DAVID I. ABRAMSON AND JOSEPH WEINSTEIN[1]

WITH THE TECHNICAL ASSISTANCE OF PEARL KRAMER

From the Department of Physiology, Long Island College of Medicine, Brooklyn, N. Y.

Received for publication November 26, 1934

Recent investigations in the field of electrocardiography have been directed toward methods for the more accurate detection and localization of myocardial lesions. Since the three conventional leads may at times fail to detect alterations consequent to a lesion, they have of late been supplemented by other leads which lie in different planes (Wolferth and Wood, 1, 2, 3), Hofman and Delong (4) and Wilson and his collaborators (5, 6, 7)). However, it has been established that myocardial lesions may exist without producing electrocardiographic alterations even in these chest and precordial leads. In the present work we have continued the investigation of a method previously reported by one of us (W.8) in which long linear electrodes are placed on the chest wall, anteriorly and posteriorly, not over the heart (as is the case with the other chest leads) but parallel to its lateral borders. By means of various combinations of these electrodes we have endeavored to introduce other recording planes in the hope that at least one might be more receptive to electrical changes of small magnitude than are the three standard leads or lead IV. This report deals with the results obtained from the application of the above method in the cat, following the production of experimental lesions by the cautery.

METHOD. The experiments reported below were performed on cats under an ether-chloretone anesthesia. The three conventional lead electrocardiograms and generally lead IV were taken as well as the experimental chest leads. In nine instances precordial leads were also recorded. The electrodes consisted of single strands of German silver wire ($\frac{1}{32}$ inch in diameter) moistened with a saturated solution of sodium chloride and inserted under the skin. Those utilized in our experimental leads[2] were

[1] From the Medical Service of the Jewish Hospital of Brooklyn.

[2] The electrodes used in lead IV varied from the others since each wire was formed into a helix (outer diameter, $\frac{3}{4}$ inch). The anterior electrode was inserted at the point of maximum apex impact and the posterior one directly opposite on the back;

placed at the estimated borders of the heart both anteriorly and posteriorly, as illustrated in figure 1, a and b. The electrodes were led to a selector switch whereby any chosen pair could be connected to the standard lead I contacts of the galvanometer. In the experimental leads, the right arm lead wire of the electrocardiograph was attached to one of the posterior chest electrodes and the left arm lead wire to one of the anterior electrodes. By means of such an arrangement the complexes in the experimental leads were generally similar in direction to those in the three standard leads but opposite to the ones in lead IV. In the precordial leads the active electrode on the anterior chest wall was likewise connected with the left arm lead wire and the indifferent electrode on the left leg with the right arm lead wire.

After the electrodes were attached, a tracheal cannula was inserted and artificial respiration instituted. The chest and pericardial sac were entered from either the right or left side depending upon which ventricle was to be traumatized, special care being taken not to disturb the position of the electrodes. An electric cautery was employed to injure a definite area on the epicardial surface of the heart in each case, according to the method used by Crawford et al. (9). In the first experiments the burn was purposely large, covering an area of about $\frac{1}{2}$ cm. in diameter and extending as deeply as possible into the muscle substance without entering the ventricular cavity. In the remainder of the cases, very small and superficial cauterizations were made. In some of the latter, following the disappearance of the electrocardiographic changes previously induced, the heart was again cauterized but in a different site.

In the first four experiments, one set of tracings was taken before the chest was opened and a second set just before cauterization. Since both appeared generally to be similar, it was thought unnecessary to continue this procedure. In the remaining cases, control electrocardiograms of limb as well as chest leads were recorded with the chest open and artificial respiration instituted. After the production of the injury, another set of tracings was taken; the standard leads and lead IV first, and then the experimental chest leads. The latter were designated by the first letters of the two electrodes in each combination, the ones situated posteriorly bearing a prime to differentiate them from those anteriorly. The following combinations were employed (fig. 1, c and d):

R′R—right posterior and right anterior electrodes
L′L—left posterior and left anterior electrodes

---

their connection with the electrocardiograph being similar to that suggested by Wolferth and Wood (1), i.e., the right arm lead wire was attached to the anterior electrode and the left arm lead wire to the posterior electrode.

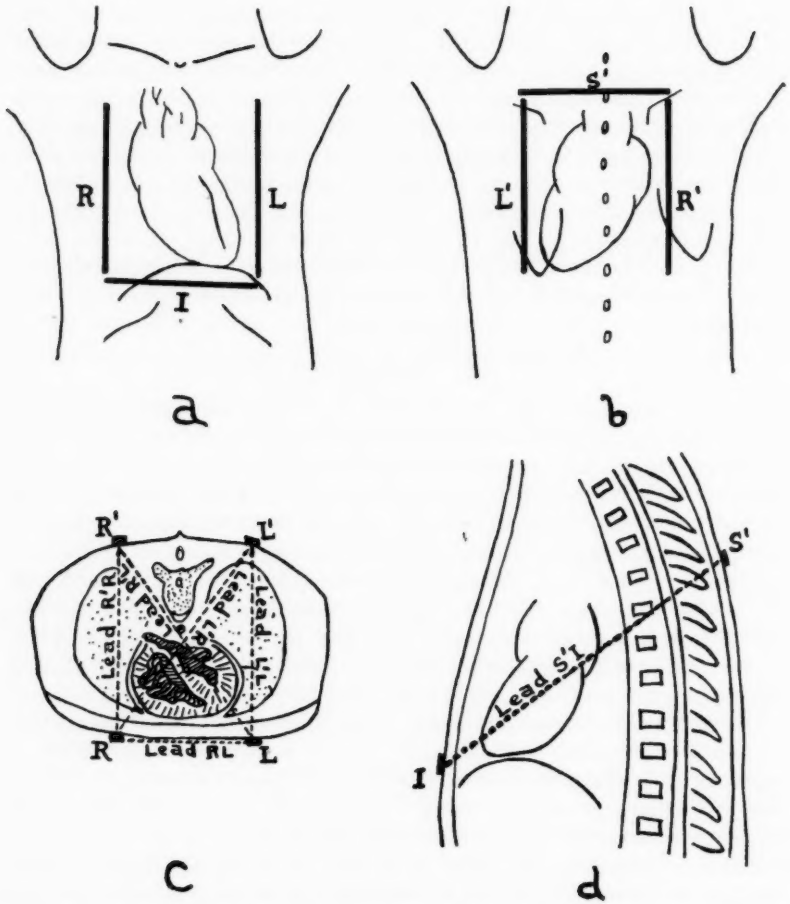


Fig. 1a and b. Diagrammatic representation of the position of the experimental chest electrodes. a. Anterior surface. *R*, right electrode located some distance to the right of the sternum and extending from the second to the seventh rib. *L*, left electrode, extending in the left anterior axillary line, for the same distance and parallel to electrode *R*. *I*, inferior electrode inserted somewhat above the level of the ensiform cartilage and extending across the chest. b. Posterior surface of chest. *S'*, superior posterior electrode, extending across the back at about the level of the superior border of the scapulae. *L'*, left posterior and *R'*, right posterior electrodes, placed so as to correspond in position to *L* (left) and *R* (right) anterior electrodes respectively. c, cross section and d, lateral view of the chest illustrating the various electrode combinations. *R'R*, right posterior and right anterior electrode. *L'L*, left posterior and left anterior electrode. *RL*, right anterior and left anterior electrode. *S'I*, superior posterior and inferior anterior electrode. *R'L*, right posterior and left anterior electrode. *L'R*, left posterior and right anterior electrode.

RL—right anterior and left anterior electrodes[3]
S'I—superior posterior and inferior anterior electrodes
R'L—right posterior and left anterior electrodes
L'R—left posterior and right anterior electrodes

At the end of each experiment (25 in all) the heart was removed, examined for size, position and depth of lesion, and preserved in alcohol.

Detection of myocardial lesions with the standard leads, lead IV and the experimental chest leads. Before analyzing the electrocardiographic results obtained by cauterization of the various portions of the heart, it is necessary to describe first the characteristics of the normal tracing for each of the experimental chest leads. With the exception of the amplitude of the deflections, the records obtained with any one lead were grossly similar to one another and differed little from those of the other experimental chest leads (figs. 2a and 3a). The P wave was usually upright and of low voltage, the R deflection of moderate size, S of small or moderate size and T upright. Tracings of lead RL, however, frequently displayed a Q wave and no S, with a T deflection that was sharply inverted. In the other leads (with the exception of one tracing of L'R) a Q wave was not present. The P wave in L'R was found to be inverted in three instances, and similarly the T wave in R'L. All experiments in which an elevation or depression of the RS-T segment was seen before cauterization, possibly as a result of the operative trauma, were discontinued unless the changes disappeared in a short time.

Following the experimental production of a lesion, the various electrocardiograms were studied primarily for RS-T segment or T wave alterations. Aside from the first four experiments in which the injury was intentionally extensive, it was our aim to make the lesions small enough to produce the minimal effect on the RS-T interval in the conventional leads and lead IV. Despite this precaution, in a number of cases alterations were observed either in the standard leads, lead IV, or in both. The changes in the standard leads consisted of either an elevation of the RS-T interval in leads I and II (T_1 type), a similar change in leads II and III (T_3 type), or an elevation in all three leads (not classifiable according to the groupings suggested by Parkinson and Bedford, 10). Lesions on the anterior surface of the left ventricle produced changes of the RS-T segment of the three standard leads in four out of seven instances; lesions on the posterior surface in four out of six instances. Injury to the anterior surface of the right ventricle resulted in an alteration of the RS-T interval in only one out of seven cases; injury to the posterior surface, in three out of five cases. Changes in lead IV consisted either of an elevation or depres-

[3] In this lead the right anterior electrode was connected with the right arm lead wire of the electrocardiograph and the left anterior electrode with the left arm lead wire.

sion of the RS-T interval. In the three instances in which this lead was recorded when the anterior surface of the left ventricle was cauterized, all records showed a depression of the RS-T interval. Lesions on the posterior surface of the same ventricle likewise produced a depression of the RS-T segment in two cases, while one remained normal. Injury to the right ventricle anteriorly resulted in a depression of the RS-T segment in lead IV in three out of six cases, the remaining three showing no change.

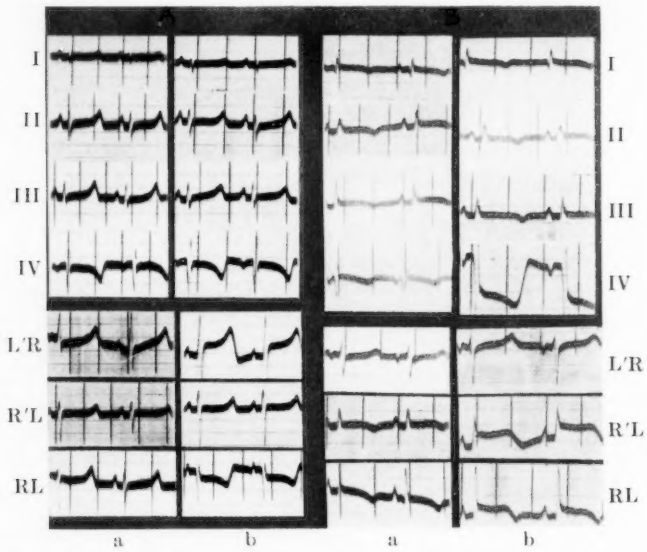


Fig. 2. Lesions on the anterior surface of the heart.

A, right ventricle. a, normal tracings. b, tracings taken after cauterization. No changes seen in leads I, II, III and IV. An RS-T segment elevation noted in L'R and a depression in RL. R'L shows no abnormalities.

B, left ventricle. a, normal tracings. b, tracings taken after cauterization. Leads I, II, and III are normal, while lead IV shows a depression of the RS-T segment. An elevation of the RS-T interval noted in R'L and RL, while L'R shows no abnormalities. One centimeter equals 1 millivolt. Time 0.2 second.

A lesion on the right ventricle posteriorly produced an elevation of the RS-T segment in two instances, a depression in one, and no change in the other two. In every experiment two or more of the experimental chest leads showed a change of the RS-T segment. The type of change and the leads affected in each case will be discussed under the section on localization of lesions.

Relative worth of the standard leads, lead IV and the experimental chest

leads in detecting myocardial lesions. In our experience, the electrocardiographic changes resulting from cauterization of small areas were transient as a rule. For this reason, the standard leads and lead IV were recorded immediately after injury, and then the experimental chest leads in order to rule out the possibility that the absence of change in the standard leads and lead IV was due to the transitory nature of the disturbance produced. Examination of our results shows that even with this precaution, in only twelve out of the twenty-five experiments were alterations of the RS-T

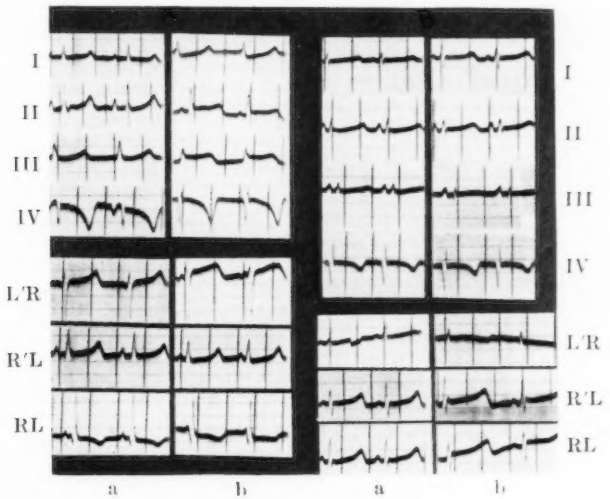


Fig. 3. Lesions on the posterior surface of the heart.

A, right ventricle. a, normal tracings. b, tracings taken after cauterization. A depression of RS-T segment noted in lead I and an elevation in leads II and III, with no change in lead IV. An elevation of the RS-T segment noted in L'R and a depression in RL. R'L shows no abnormalities.

B, left ventricle. a, normal tracings. b, tracings taken after cauterization. Leads I, II, III and IV show no changes. An RS-T elevation noted in R'L and RL, with no change in L'R.

segment observed in the three standard leads, and in eleven out of seventeen in lead IV. Of the remaining six experiments which showed no change in lead IV, in five of these the conventional three leads likewise remained normal. It is significant, therefore, that whether or not the three standard leads or lead IV demonstrated a change characteristic of the presence of an experimental infarct, two or more of the experimental chest leads in each instance consistently showed an RS-T segment alteration.

Localization of myocardial lesions with the experimental chest leads. Ex-

amination of table 1 reveals that a lesion on either the anterior or posterior surface of a ventricle produced the same type of change in practically the same leads in each case. In the presentation of the data on localization, therefore, the results will be classified according to the site of the cauterization on one or the other ventricle, with no designation of the surface of the heart involved. A number of the experimental chest leads (R′R, L′L and S′I), although generally demonstrating a change of the RS-T segment, did not contribute toward specific localization, and accordingly will not be discussed below. The results obtained with them are, however, incorporated in table 1.

TABLE 1

Localization of lesions by means of the experimental chest leads

EXPERIMENTAL CHEST LEADS	ANTERIOR SURFACE TYPE OF RS-T CHANGE				POSTERIOR SURFACE TYPE OF RS-T CHANGE			
	Total number of cases	Elevation	Depression	No change	Total number of cases	Elevation	Depression	No change
Left ventricle								
R′L	9	9	0	0	6	5	0	1
L′R	3	0	1	2	4	0	0	4
RL	7	6	0	1	6	5	0	1
R′R	4	3	0	1	4	0	0	4
L′L	6	6	0	0	6	3	0	3
S′I	7	7	0	0	3	2	0	1
Right ventricle								
R′L	5	1	0	4	3	0	0	3
L′R	5	5	0	0	5	5	0	0
RL	5	0	4	1	3	0	3	0
R′R	5	5	0	0	3	2	0	1
L′L	5	1	0	4	3	0	0	3
S′I	5	3	0	2	3	1	0	2

In the left ventricle a lesion, located either anteriorly or posteriorly, produced an elevation of the RS-T segment in lead R′L in all tracings but one and in lead RL in eleven out of thirteen cases, while the RS-T interval in lead L′R remained normal or became somewhat depressed. In the case of the right ventricle the reverse is true; a lesion on either surface generally produced no change of the RS-T segment in lead R′L (seven out of eight experiments), a depression of the RS-T interval in lead RL (seven out of eight experiments) and a consistent elevation in lead L′R. While the T_1 or T_3 type of change in the standard leads is of some use in localizing lesions to the anterior or posterior surface of the heart (9, 11, 12), it would appear

from the above data that in the cat the experimental chest leads localize the injury to one or the other ventricle.[4]

Relative position of the electrodes to the heart. According to Wilson and his collaborators (5, 6, 7) more accurate results in the detection of myocardial lesions are obtained with precordial leads[5] than with chest leads. In order to investigate this point in the cat, in nine instances two precordial leads were taken as well as the experimental chest leads; an electrode on

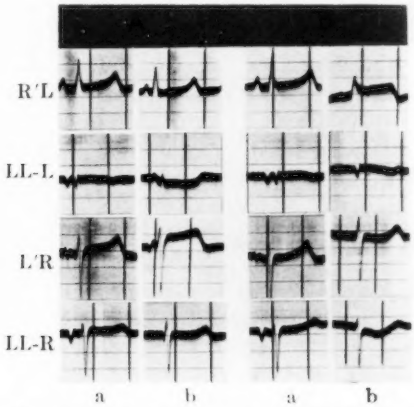


Fig. 4. Comparison of tracings obtained with chest leads R'L and L'R and the precordial leads LL-L and LL-R respectively. The position of the anterior chest electrode remained the same in both the chest and in the corresponding precordial lead, the only difference between the two being the position of the other electrode in the combination. In the precordial lead, an electrode on the left leg was substituted for the posterior chest wire in the chest lead.

A, cauterization of right ventricle posteriorly. a, normal tracings. b, tracings after cauterization. An elevation of the RS-T segment is noted in the experimental chest lead L'R, with no change in the corresponding precordial lead LL-R. Slight depression of RS-T segment in precordial lead LL-L.

B, cauterization of the left ventricle posteriorly. a, normal tracings. b, tracings after cauterization. An elevation of the RS-T segment is noted in the experimental chest lead R'L with no change in the corresponding precordial lead LL-L. Slight depression of RS-T segment in precordial lead LL-R.

[4] Two experiments on localization were attempted in the dog, in one of which the left ventricle anteriorly was cauterized and in the other the right ventricle anteriorly. In the former, after the production of the injury, an elevation of the RS-T interval was noted in leads R'L and RL, with no change in L'R. In the latter, an elevation of the RS-T segment was observed in lead L'R, a depression in RL and no change in lead R'L. Accordingly, these results are similar to those in the cat.

[5] In precordial leads, one electrode is placed on the precordium and the other at some distant point from the heart.

the left leg being substituted for the posterior chest wire in each of the two combinations, R′L and L′R. When relatively large lesions were produced, the anticipated change was usually present in both the experimental chest lead and in the corresponding precordial lead (although not as marked in the latter). In the case of small cauterizations, especially when they were posteriorly situated, the precordial leads were much less receptive to changes than were the experimental chest leads. In fact, in five out of the nine cases, following the production of a lesion, the precordial leads appeared unaffected, while the RS-T segment of the experimental chest leads was definitely elevated (fig. 4). However, not alone was it of advantage to place both electrodes on the chest, but the most accurate results were obtained only when certain combinations of these electrodes were used. Examination of table 1 reveals that the lead in which a right posterior and left anterior electrode were combined (R′L) as well as the one utilizing a left posterior and right anterior electrode (L′R) almost consistently showed characteristic changes of the RS-T segment. Lead RL, in which both electrodes were placed on the anterior chest wall, was likewise of considerable value. On the other hand, the changes observed in the remaining chest leads were not specific or constant enough to contribute appreciably to the data obtained with the above leads alone. It would, therefore, appear that in the cat, the proximity of *both* electrodes to the lateral borders of the heart and the combinations of electrodes used are important in the detection and localization of experimental lesions.

SUMMARY

A study was made to determine the sensitivity of certain chest leads in the detection and localization of small experimental ventricular lesions in the cat. These leads consisted of combinations of linear electrodes placed parallel to the lateral borders of the heart anteriorly and posteriorly. In all twenty-five experiments, following the production of the lesion by the cautery, an RS-T segment alteration was observed in at least two or more of these chest leads. In only twelve of these experiments (twelve out of the twenty-five) were similar changes observed in the three standard leads, while deviations from the normal in lead IV were present in eleven out of seventeen of the above cases. Using certain of the experimental chest leads the lesion could be accurately localized to one or the other ventricle.

From the above, it would appear that the experimental chest leads are more receptive in detecting and localizing experimentally produced lesions in the cat than are the three standard leads or lead IV.

REFERENCES

(1) Wolferth, C. C. and F. C. Wood. Am. J. Med. Sci **183:** 30, 1932.
(2) Wood, F. C. and C. C. Wolferth. J. Clin. Invest. **11:** 815, 1932.

(3) Wood, F. C. and C. C. Wolferth. Arch. Int. Med. **51**: 771, 1933.
(4) Hoffman, A. M. and E. Delong. Arch. Int. Med. **51**: 947, 1933.
(5) Wilson, F. N., A. G. Macleod and P. S. Barker. Am. Heart J. **7**: 207, 1931.
(6) Wilson, F. N., A. G. Macleod and P. S. Barker. Am. Heart J. **7**: 305, 1932.
(7) Wilson, F. N., A. G. Macleod, P. S. Barker, F. D. Johnston and L. L. Klostermeyer. Heart **16**: 155, 1933.
(8) Weinstein, J. Ann. Int. Med. **7**: 1503, 1934.
(9) Crawford, J. H., G. H. Roberts, D. I. Abramson and J. C. Cardwell. Am. Heart J. **7**: 627, 1932.
(10) Parkinson, J. and D. E. Bedford. Heart **14**: 195, 1927–29.
(11) Barnes, A. R. and M. B. Whitten. Am. Heart J. **5**: 142, 1929.
(12) Haney, H. F., M. C. Borman and W. J. Meek. This Journal **106**: 64, 1933.

THE ESTROUS CYCLE AND WEIGHTS OF ORGANS IN RELATION TO THE HYPOPHYSIS IN THE HAIRLESS RAT

FREDERICK E. EMERY

From the Physiology Department, University of Buffalo

Received for publication November 21, 1934

Within the last decade a strain of hairless rats has been developed in the Animal Husbandry department at the University of Illinois. The hairless condition is a recessive character and is transmitted according to Mendelian inheritance (Roberts, 1924, 1926). The hairless adult male is quite fertile but not as aggressive toward the female as the albino male. In contrast to this the hairless female rarely becomes pregnant and when young are born she does not lactate properly and the litter dies. In order to keep the colony going heterozygous females are mated to hairless males; one-half of the young born are homozygous and hairless and half are heterozygous and haired.

The writer was interested in a physiological or hormonal explanation of the hairless condition and the lack of fertility in the female. Therefore a few years ago some rats were obtained from Doctor Roberts and a colony started. The data obtained on the estrous cycles, weight of organs and anterior pituitary sex hormones are described in this paper.

METHODS AND RESULTS. *The estrous cycles.* Daily (morning) vaginal smears were made by the pipette method. All rats were kept in the same room and given the same food and care. Forty-five of the sixty hairless females started on the estrous cycle experiment lived four months or longer. The data from these have been arranged in table 1 to show the mean length of the estrous cycles for each thirty days of age. As shown the length of the cycles varies from 8.2 to 13.8 days; this is about twice the length characteristic of the albino and heterozygous groups. These average figures for each month of age do not include the cycles that were longer than thirty days. Such long periods of diestrus are rarely found in albino rats or in the heterozygous haired females of the hairless strain but are veries common in the hairless females. In the latter 14 per cent of the cycles were thirty days or longer in length. In fact it is not uncommon for a young hairless female to be in estrus a few times and then continue in a diestrous period for several months followed again by one or more estrous cycles or by death. Some of the hairless females were at times in heat for shorter intervals. A more detailed study of the data in table 1 showed that cycles

of six days or less in length occurred in 85 per cent of the cycles of the albino rats, 87 per cent of the heterozygous haired group and 40 per cent of the hairless group. A series of consecutive cycles five or six days in length although occurring in hairless individuals was not continued for long intervals as in normal rats. The duration of the estrous phase in hairless rats was, as far as could be determined, normal in length.

Weights of organs. The long diestrous periods of the hairless rats suggested an abnormal function and weight of the endocrine glands concerned with estrus. Weights were obtained for the various organs and compared to those of the albino rats reared during the same period on the same type

TABLE 1

The average length of the estrous cycles for normal albino, heterozygous and hairless rats is given according to age

GROUP	NUMBER OF RATS	ESTROUS CYCLES—AGE IN MONTHS											
		1	2	3	4	5	6	7	8	9	10	11	12
Normal albinoes	16		5.4	5.1	4.9	4.9	4.5	4.6	4.8	6.0	6.4	6.0	5.9
Heterozygous	20		7.6	6.4	4.7	4.9	5.5	5.5	4.6	5.3	5.3	5.0	5.5
Hairless*	45		10.5	13.0	9.7	13.7	10.8	8.2	13.8	11.4	12.7	10.8	12.6

* The mean age for opening of the vaginal canal was 46 days.

TABLE 2

Body weights in grams, other weights in milligrams. The females are virgins

GROUP	NUMBER OF RATS	BODY WEIGHT	KIDNEYS	SPLEEN	PITUITARY	THYROIDS	ADRENALS	TESTES
Normal albinoes	10	210	1700	690	7.5	15.8	32.0	2350
Heterozygous	15	205	1927	832	7.2	12.5	39.2	2567
Hairless	15	215	2070	610	8.0	14.4	48.1	2475
				UTERUS				OVARIES
Normal albinoes	20	165	1315	320	9.0	14.6	42.0	45.0
Heterozygous	12	168	1573	345	9.7	12.6	50.0	49.0
Hairless	20	170	1750	115	8.0	14.0	52.0	39.0

of food. The kidneys and adrenals are larger, the ovaries and uterus smaller, in hairless rats (table 2). The other organs do not differ in the hairless or in the heterozygous groups from the weights given for the same sex in albino rats. The differences shown in table 2 for the spleen, pituitary, thyroids and testes in the several groups are considered to be within the normal range of variation and without significance. The weights of the kidneys and adrenals in heterozygous rats being midway between the albino and hairless groups is perhaps to be expected if the hairless condition is associated with large kidneys and adrenals. The adrenal glands are known to be larger in many wild species of rats (Freudenberger, 1932), and

since this hairless colony was developed from a wild strain, this might explain the large adrenal glands. The small size of the ovaries and uterus in the hairless females is probably secondary to the pituitary and thyroids although the two latter are normal in weight. The ovaries and uterus respond well to pituitary grafts by an increase in weight, thus again suggesting that the small size of these organs is secondary to the pituitary gland. The ovaries of untreated hairless rats were found by histological examination to be full of corpora lutea and quite normal in appearance. From these observations and a study of the weights of the organs it seems, therefore, reasonable to conclude that the pituitary-ovarian relationship is not functioning normally in hairless rats and as a result the estrous cycles are depressed.

The comparative function of the hypophysis. As mentioned in a previous section the weights of the ovaries and uterus of the hairless females suggest a pituitary deficiency. The amount of gonadal stimulating hormone in the pituitary gland has been determined by the usual implant method. The donors were normal and castrated rats of both sexes in the albino, heterozygous and hairless groups. The results obtained are shown in table 3. One pituitary, from the albino male rat, is about the minimal dose given as an implant that will cause the vaginal canal to open and the ovaries to increase in weight. When this minimal dose was used, both the heterozygous and hairless males were found to have more potent pituitaries than the albino males; this is made clear by a comparison of the ovarian weights of the recipients which are 21.0, 26.0 and 17.5 mgm. (table 3). These differences, though difficult to understand, nevertheless do show that the males of this strain of wild rats have more potent pituitary glands than do those of our albino colony.

In cases where virgin female rats are used as donors four pituitary glands are required to bring about changes in the ovaries of the recipients. In the females also, as was found with males, the hairless rats gave the most potent hypophyses, with the heterozygous females intermediate in position. This is made clear by comparing the weights of the ovaries of the recipients. The means are 20.2, 17.4 and 16.6 mgm. in the hairless, heterozygous and albino groups (table 3). The tendency for the ovaries and especially the uteri to be somewhat atrophic in the hairless females may logically be considered as a condition of hyposecretion of theelin. The lessened amount of theelin in the circulation will result in a greater potency of the pituitary gland. This explanation is not applicable to the heterozygous females in which weights of the reproductive organs and estrous cycles are normal. So here again, as was suggested for the heterozygous and hairless males, the more potent pituitary glands may be characteristic of this wild strain of rats and not linked with the factors concerned with hairlessness.

The heterozygous males and the hairless males have shown after castra-

tion the usual increase in the sex hormone of the hypophysis. The figures for both ovarian weights and percentages shown in table 3 for these groups are well within the range characteristic of castrated albino rats. On the other hand, when castrated hairless females are used as donors the picture is different in that the usual increase in the anterior pituitary sex hormone is largely absent. This is best shown by comparing 353 per cent to 106 per cent which represents the percentage increase in the ovarian weights of recipients implanted with glands from castrated albino and hairless females (table 3). Also as shown in table 3 the pituitary glands of heterozygous

TABLE 3

The effect of pituitary implants from several types of donors on the weight of the ovaries of albino immature rats is shown

Implants given at 25 days of age; also on the following day when more than one implant was given. Ten to 20 rats in each group.

DONORS			RECIPIENTS—KILLED AT 30 DAYS OF AGE				
Type	Number to each recipient	Body weight	Body weight	Weight of ovaries			
				High	Low	Mean	Per cent increase*
							per cent
Males:							
Normal albinoes	1	205	48.5	37	10	17.5 ±1.8	16.6
Heterozygous	1	205	48.5	40	10	21.0 ±1.8	40.0
Hairless	1	198	42.1	63	9	26.0 ±2.7	73.3
Castrated albinoes	1	187	42.8	100	43	60.9 ±3.9	306
Heterozygous castrated	1	190	46.4	94	27	53.4 ±6.2	276
Castrated hairless	1	191	49.0	144	18	68.2 ±9.8	353
Virgin females:							
Normal albinoes	4	178	49.4	22	12	16.6 ±0.5	10.6
Heterozygous	4	175	50.0	23	13	17.4 ±1.0	16.0
Hairless	4	165	52.0	26	16	20.2 ±1.3	34.6
Castrated albinoes	1	181	51.6	163	25	68.5 ±9.3	353
Castrated heterozygous	1	170	46.0	66	13	32.8 ±2.9	118
Castrated hairless	1	177	52.0	63	13	30.9 ±2.8	106

* To obtain these percentages the weight of the normal ovaries is taken as 15 mgm.

castrated females were not very effective in producing large ovaries in the recipients.

DISCUSSION. From our discussion so far of the estrous cycles, the organ weights and the physiology of the sex relationship in the hairless rat it is clear that this strain of rats differs in several respects from our albino colony. The rôle of the hairless condition in these disturbed functions, as for example long periods of diestrus and lack of the usual increase in the sex hormone of the hypophysis following castration in the female, is not known. The hairlessness may be the primary factor in bringing about

these abnormal changes. If this be true then the endocrine dysfunction like the hairless condition is inherited. The fact that the irregular estrous cycles have been so constantly associated with the hairless females seems to indicate a common cause. Lactation is also absent in the hairless female and the young if left with the mother die of starvation. Young strong albino rats were placed with a hairless mother that had just given birth and these also died within a few days. Milk was not seen through the abdominal wall of any of these young.

The amount of theelin normally present in the blood of female rats has been suggested by several investigators to regulate the hypophysis in such a way as to vary or control the concentration of the pituitary sex hormone in the blood. In cases of lack of theelin, as in castration, the sex hormone in the serum is sufficient in amount to give a positive reaction in immature rats (Emery, 1932). In the hairless female rats this regulatory mechanism of theelin and other hormones associated with the rhythm of the estrous cycles is not functioning properly and irregularity in the estrous cycles occurs.

SUMMARY

1. A hairless strain of rats has been studied in respect to the estrous cycles, weights of organs and pituitary gonad relationship.

2. The spleen, pituitary, thyroids and testes are similar in weight in albino, heterozygous and hairless males. The kidneys and adrenals are larger in heterozygous and hairless rats of both sexes. The ovaries and uterus are smaller in the hairless females than in heterozygous and albino females.

3. The estrous cycles are prolonged in the diestrous phase, usually irregular and often absent.

4. The hairless female may give birth to young but is unable to lactate.

5. The gonadal stimulating hormone of the hypophysis is greatest in amount in hairless males and females, intermediate in the heterozygous and least in albino rats. After castration in hairless and heterozygous females the pituitary is quite inferior to the pituitaries of castrated albino rats as judged by the implant method of testing.

6. A possible relationship between the hypophysis and the hairless condition is discussed.

REFERENCES

EMERY, F. E. This Journal **101**: 246, 1932.
FREUDENBERGER, C. B. Am. J. Anat. **50**: 293, 1932.
ROBERTS, E. E. Anat. Rec. **29**: 141, 1924.
Ibid. **34**: 172, 1926.

QUALITATIVE BLOOD CELL CHANGES IN THE RAT DUE TO VITAMIN A

PAUL D. CRIMM AND DARWIN M. SHORT

From the Research Laboratory of Boehne Tuberculosis Hospital, Evansville, Indiana

Received for publication September 24, 1934

This report covers the findings on the hemograms of 990 rats, including 369 vitamin A-deficient rats and 148 rats in various stages of hypervitaminosis A, together with 473 litter mate controls. A review of the literature revealed an absence of conclusive results on the blood of vitamin A-deficient rats.

METHODS AND STANDARDS. The minimum weight requirement for young rats at birth was 6.0 grams. All litters were reduced to eight on the fifth day. The litters were weaned uniformly at 21 days. The maximum variation in weight allowed was ±2.0 grams each within a group, and a gross variation between litters of ±10.0 grams. The rattery temperature was kept at 78°F. The animals were maintained in galvanized iron cages with wire mesh floors to prevent coprophagy. At no time during the period of these experiments was there any epizoötic in our colony.

A diet[1] containing 2 per cent cod liver oil, of uniform quality (1400 I.U. of vitamin A and 100 I.U. of D per gram), furnishes more than an adequate supply of vitamins A and D for normal growth and reproduction. The vitamin A-deficient diet was the same except for the absence of the cod liver oil, which was replaced by cottonseed oil containing vitamin D in the form of irradiated ergosterol. Distilled water and food were given *ad libitum*.

Blood was secured by tail cutting. Bleeding was always done at the same hour of the day. A gross leucocyte count was made, using the Thoma improved Neubauer method (Trenner, 1924). Each animal was bled once and discarded. The normal leucocyte count for our colony was found to range between 4,000 and 16,000 cells, with an average of about 10,000 cells.

	per cent
[1] Purified casein	18
Salt mixture (Hawks and Oser, 1931), modification of Osborne and Mendel (1917)	4
Cottonseed oil	4
Cod liver oil	2
Brewer's yeast	8
Corn starch	64

Neutrophil maturation lag in avitaminosis A. In a preliminary study we (Crimm and Short, 1934) made complete hemograms from birth to maturity on rats, which established criteria for the neutrophil cells according to age variation. In this experiment, a lesser neutrophil Arneth (1904) index (about 10 index per cent) was found in vitamin A-deficient animals at various intervals from 28 to 44 days experimental time (expts. X-1, X-2, X-3 and X-4, table 1). A study was then made to ascertain just how early this maturation lag was manifest by repeating the experiment with various

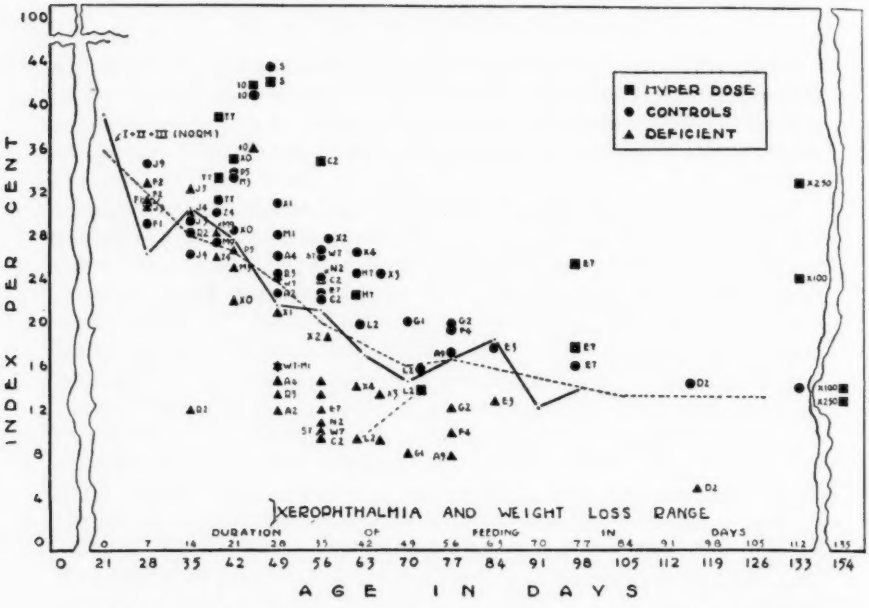


Fig. 1. Composite results of neutrophil indices in avitaminosis A, and hypervitaminosis A. Controls in these experiments checked with the line marked "I + II + III (Norm.)" determined in previous work (Crimm and Short, 1934)

vitamin A-deficient feeding periods. The composite results of the neutrophil indices are depicted in figure 1.

The means of the indices of the control and experimental groups were plotted about the norm (fig. 1). The accepted formula for standard deviation between two means

$$\epsilon = \sqrt{\frac{\sigma 2_C}{N_2} + \frac{\sigma 2_A}{N_A}}^{2}$$

[2] σC = standard deviation of index of control animals.
σ_a = standard deviation of index of deficient animals.
N = number of animals in respective experimental groups.

is used (fig. 2). A difference in excess of 3ϵ is considered significant (Mills, 1924, and Dunn, 1929). The shaded areas in this chart (fig. 2) represent the actual determined value of 3ϵ in the above formula.

The difference between the control and vitamin A-deficient mean indices becomes significant

$$\left(\sum \frac{C}{N_C} - \sum \frac{A-}{N_A} > 3\sqrt{\frac{\sigma 2}{\frac{C}{N_C}} + \frac{\sigma 2}{\frac{A-}{N_A}}}\right)^3$$

following a feeding period of 21 to 28 days. The maximum difference appeared between 35 to 42 days of deficiency (fig. 2). In experiment R-2 the vitamin A-deficient diet was placed in the whelping cages so that the young rats had no opportunity to secure any supply of vitamin A other

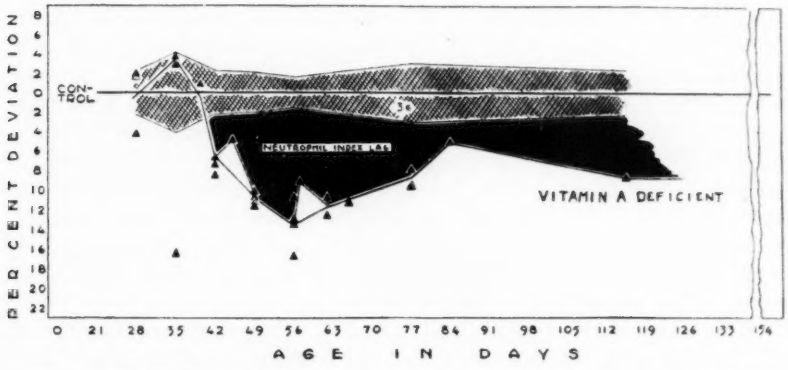


Fig. 2. Vitamin A-deficient groups with control groups indices corrected for age (to base line) with shaded area representing determined value of 3ϵ in formula.

than through the mother's milk. This group manifested its neutrophil lag at 14 days' experimental feeding. Weaning at 23 to 25 days delayed the appearance of the neutrophil index lag from seven to twenty-one days.

In this work, the first three neutrophil groups were used for the modified Arneth index. At 28 days of age (7 days of deficiency) these three groups represented about 35 per cent of all the rat neutrophil cells. At 63 days of age (42 days of deficiency) the first three neutrophil groups constituted about 15 per cent of all neutrophil cells. The maximum Arneth index lag occurs after 70 days of age (49 days of deficiency). At this stage there is a marked increase of older neutrophil cells with a highly sinuous and fragmented type of nucleus. In spite of the decline in the percentage of the

[3] Σ_C = summation of control indices.
Σ_A = summation of A-avitaminic animal indices.
N = number of animals in respective groups.

younger granulocytic cells in avitaminosis A, there is no significant gross decline in the total number of neutrophils.

Elimination of vitamin D. In a previous experiment (unpublished) a group of rats on a deficient diet were fed 0.1 cc. of vitamin D (10,000 I.U. per gram), as irradiated ergosterol, on alternate days. These rats developed a neutrophil maturation lag and the usual gross signs of vitamin A deficiency. As the subsequent experiments demanded, an equal number of vitamin D units in the form of irradiated ergosterol was supplied to either the controls or the experimental groups. This eliminated any effect from vitamin D.

Effect of halibut liver oil on the neutrophil index lag in avitaminosis A. In order to establish whether or not this lag in neutrophil delivery into the blood stream is due to the absence of vitamin A, a group of control rats (63 days old, non-deficient) and a group of deficient rats (63 days old, 42 days deficient), which evidenced significant differences in their respective Arneth indices, were fed 0.1 cc. of cottonseed oil containing vitamin D as irradiated ergosterol (equal to vitamin D units in 0.1 cc. H.L.O.), and halibut liver oil[4] respectively (expt. L-2, figs. 1 and 3). These feedings were continued for ten days. Both groups were then bled and blood counts and smears made. The gross counts revealed no significant changes, but the Arneth indices showed an increase (a "left shift"—younger cells) and a return to essentially the normal for the deficient group which had been supplied vitamin A as halibut liver oil (fig. 3). It is concluded that the response was due to a component of the halibut liver oil, most probably to the vitamin A which this oil supplied in abundance.

Elimination of vitamin E as a causal factor. Of three groups of rats, two groups were depleted of vitamin A. The litter mate controls (group 1) were maintained on the adequate diet. Two groups (groups 2 and 3) of the deficient animals received the vitamin A deficient diet, reinforced with 0.01 cc. of wheat germ oil, on alternate days thereafter (21 days feeding time), so as to assure an adequate intake of vitamin E. Counts and smears taken at 42 days experimental time showed the controls (group 1) to have 19.2 index per cent, and the deficients plus vitamin E (groups 2 and 3) to have 12.4 index-percent. Thus, the deficient animals showed a lag in the neutrophil index despite the supplement of vitamin E.

Prolonged leucocytic response to foreign protein in avitaminosis A. Twenty-eight rats (21 days old) were divided into three groups. Group 1 was maintained on the control diet containing 2 per cent cod liver oil. Group 2 was maintained on a deficient diet 42 days. Group 3 was given a deficient diet for 21 days and then fed halibut liver oil, 0.1 cc. *per diem*

[4] One-tenth cubic centimeter serum pipette actually delivers 0.0828 gram of oil. The halibut liver oil contained 50,000 I.U. of vitamin A per gram, but only 1,200 I.U. of vitamin D per gram.

(4,000 I.U. vitamin A). After all groups were on their respective diets 42 feeding days (63 days of age) leucocyte counts and blood smears were made. Following the blood counts 0.5 cc. of *B. typhosus* vaccine was given intraperitoneally. Six hours later counts and smears were repeated. It was noted that the groups made deficient in vitamin A had higher initial counts than the controls. This was true of both experimental groups 2 and 3. Following the injection of the foreign protein the vitamin A-deficient animals had a more persistent leucocytosis than did either the controls

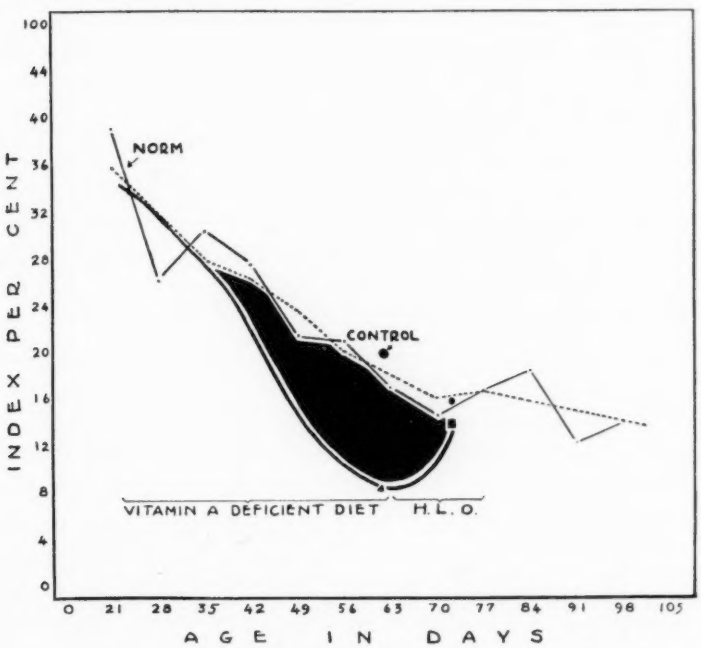


Fig. 3. Showing that the neutrophil index lag returns to normal after the administration of vitamin A as halibut liver oil.

or group 3, whose diet had been supplemented by vitamin A in the form of halibut liver oil. A duplication of the previous experiment on a second series of 32 animals was made. Again there was persistent leucocytosis following foreign protein injection in the deficient group. The body response to foreign proteins is prolonged because the reticulo-endothelial tissues in deficient animals are less able to fix and dispose of foreign protein material.

Hypervitaminosis A in the rat. The converse of the preceding series of

vitamin A-deficiency experiments was done, using the same methods and standards, but administering excessive dosages of vitamin A. The findings are herein reported on the observations and hemograms of 114 controls and 148 experimental rats (expts. T-T, X-0, 10, S, C-2, H-7, E-7, X-100 and X-250, fig. 1). The controls were fed the control diet (containing 2 per cent cod liver oil) and additional vitamin D in the form of irradiated ergosterol. In addition to this control diet (minus the irradiated ergosterol), the experimental groups also were fed either standardized halibut liver oil (50,000 I.U. of vitamin A per gram; 1,200 I.U. of vitamin D), or a "vitamin A concentrate" (420,000 I.U. vitamin A per gram; 7,400 I.U. of vitamin D per gram). This concentrate was prepared by saponification of fish liver oils. It was standardized spectrophotometrically and checked biologically for its vitamin A content.

Hemograms made following the prolonged feeding of this particular "vitamin A concentrate" revealed markedly anemic animals (color index 1.04 to 1.07), with a significant leucocytosis. There was a relative increase in the neutrophil per cent and a "left shift" of the Arneth index, with a decrease in the older Arneth groups (groups 4 and 5). The blood fragility range, as determined by the method of Griffin and Sanford (1918), was extended in the lower concentration range. This indicated that the bone marrow activity had not decreased, but that the rate of destruction of the older blood cells had increased. The latter effect was also evidenced in the increased number of reticulocytes.

The administration of vitamin A (27,000 to 100,000 international units) as halibut liver oil showed that the Arneth indices of the halibut liver oil groups were not significantly different from those of the control groups (expts. T-T, H-7, 10 and E-7, fig. 1).

After a prolonged feeding of "vitamin A concentrate" (210,000 to 420,000 I.U. of vitamin A), the organs are blanched and bloodless. All serous and mucous membranes are pale. The thyroid is enlarged. Cyst-like areas (0.5 to 1.0 mm.) are scattered throughout both lungs. These extend above the lung surface. The spleen is greatly reduced in size. The testes are soft and flabby, with gross injection of the epididymis. These findings essentially duplicate and substantiate those of Drigalski (1933) and Collazo and Rodriguez (1933), except for the absence of the spontaneous fractures reported by the latter.

Another group of rats were fed 1.0 gram of a second "vitamin A concentrate" (585,000 I.U. vitamin A per gram, 6,400 I.U. vitamin D per gram) within one week. These animals died a few days later from dehydration, due to inanition and diarrhea; and without leucocytosis. Rats which received within a week's time 200,000 I.U. of this same "vitamin A concentrate" lost weight, but continued to eat and drink normally. They returned to normal.

Effect of desiccated thyroid on vitamin A deficient rats. Deficient rats which were given 65.0 mgm. of desiccated thyroid per day for five days showed xerophthalmia 11.7 days (±2.3 days) earlier than did litter mate deficient rats. The appearance of xerophthalmia was 100 per cent in those animals receiving the thyroid. Loumos (1934) is of the opinion that cod

TABLE 1

Blood cholesterol findings in avitaminosis A, hypervitaminosis A and controls

NUMBER OF ANIMALS	AGE	TYPE EXPERIMENT	BLOOD CHOLESTEROL
	days		*mgm. per 100 cc.*
16	55	Controls (Normal Diet, 2 per cent C.L.O.)	62
15	55	Hypervitaminosis A (H.L.O., 80,000 I.U. Vit. A)	58
10	55	Hypervitaminosis A ("Vit. A Conc.") (210,000 I.U. Vit. A)	84
16	91	Controls (Normal Diet, 2 per cent C.L.O.)	161
12	91	Vitamin A Deficient	297

All animals placed on experimental diet at 21 days.

TABLE 2

Vitamin A content of rat livers

AGE	FEEDING AND NUMBER OF ANIMALS	NON-SAPONIFIABLE MATERIAL	UNITS OF VITAMIN A PER GRAM NON-SAPONIFIABLE MATERIAL	GRAMS LIVER	INT. UNITS PER GRAM LIVER
		gram			
Weanling	Mother's Milk (17)	0.3941	1,052	36.0	290.3
35 days old	Controls (6) (2 per cent cod liver oil)	0.2640	3,460	57.8	600.0
55 days old (34 days) (deficient)	Vit. A Deficient (6) (with neutrophil lag)	0.2516	1,142	33.1	346.0
55 days old	Controls (6) (2 per cent cod liver oil)	0.4854	7,375	48.9	1,518.0
55 days old	*H.L.O. (6) (80,000 I.U.)	0.4912	2,870	59.9	5,870.0
55 days old	*"Vit. A Conc." (4) (242,000 I.U.)	0.6615	53,200	28.0	19,000.0

Controls placed on diet at 21 days of age.

* These two groups were on the control diet for 35 days prior to administration of halibut liver oil and "vitamin A concentrate," respectively.

liver oil, and perhaps vitamin A or D, has an inhibitory or protective reaction against experimental hyperthyroidism.

Blood cholesterol in hypervitaminosis A and avitaminosis A. Blood cholesterol studies were made using the method of Myers and Wardell (1918). Determinations were made in duplicate on the pooled blood of control

and experimental groups (table 1). In this experiment (table 1) animals on the control diet received about 2.0 mgm. of cholesterol per day. The animals which received halibut liver oil received about 8.0 mgm. of cholesterol per day. The blood cholesterol was not increased by the cholesterol of the halibut liver oil. The animals administered "vitamin A concentrate" received a very small fraction of cholesterol, which was not responsible for the increased Arneth index and subsequent leucocytosis. The difference in age between the two control groups may account for the difference in blood cholesterol. The blood cholesterol for each group was within normal limits. However, the hemograms for each group (table 1) corresponded to the blood findings previously noted.

Vitamin A content in the livers of the rats used in these experiments. In order to correlate our previous findings with the actual vitamin A content of the rat livers, spectrophotometric analyses (For. Letters, J. A. M. A., 1934, 103-353) were performed on the livers of the rats at various stages and on various diets (table 2). The lesser liver findings in weanlings would indicate other tissue storage of vitamin A in young rats, or a different acquisition from the mother's milk or stock ration prior to weaning. The hemograms of the controls, deficient and hypervitaminosis A animals correlated with their respective vitamin A liver contents.

SUMMARY

1. In these experiments on vitamin A, the possibility of effects from vitamins D and E and the water-soluble vitamins has been under control.

2. A neutrophil index lag (modified Arneth index) was repeatedly found to be an early manifestation of the cellular changes associated with vitamin A deficiency in the rat.

3. The restoration of the normal neutrophil index, following the administration of vitamin A as halibut liver oil, furnishes additional evidence that vitamin A is essential for normal cellular generation and function.

4. The absence of vitamin A inhibits the formation of granulocytic cells (qualitative phase, "right shift"). These blood cells become extremely senile in type. Despite the decline in the percentage of younger granulocytic cells in avitaminosis A, there is no significant gross decline in the total number of neutrophils.

5. The function of the reticulo-endothelial system to fix and dispose of foreign protein is impaired in avitaminosis A.

6. Normal hemograms were obtained from normal animals after the administration of vitamin A as halibut liver oil. Following a prolonged administration of "vitamin A concentrate," a leucocytosis occurred with a "left shift" of the Arneth index.

7. A-deficient rats, fed desiccated thyroid, showed xerophthalmia approximately 11 days earlier than litter mate deficient animals.

8. The blood cholesterol of rats receiving halibut liver oil was found to be within normal limits. The cholesterol of the fish oil is not responsible for the increased Arneth index ("left shift") and accompanying leucocytosis of hypervitaminosis A.

9. The occurrence of certain gross pathological changes in hypervitaminosis A is confirmed.

10. Spectrophotometric determinations of the vitamin A content of rat livers were found to correlate blood cell and pathological findings.

REFERENCES

ARNETH, J. Neutrophilen weissen Blütkörperchen bei Infections-Krankheiten Fischer, Jena, 1904.
COLLAZO, J. A. AND J. S. RODRIGUEZ. An. de med. int. **2**: 291, 1933.
CRIMM, P. D. AND D. M. SHORT. This Journal **108**: 324, 1934.
DRIGALSKI, W. Klin. Wchnschr. **8**: 308, 1933.
DUNN, H. L. Physiol. Rev. **9**: 275, 1929.
GIFFIN, H. E. AND A. H. SANFORD. J. Lab. Clin. Med. **4**: 465, 1918.
HAWKS, P. B. AND B. L. OSER. Science **74**: 471, 1931.
LOUMOS, S. Proc. Soc. Exp. Biol. and Med. **31**: 895, 1934.
MYERS, V. C. AND E. L. WARDELL. J. Biol. Chem. **36**: 147, 1918.
MILLS, F. C. Statistical methods. New York, 1924.
OSBORNE, T. B. AND L. B. MENDEL. J. Biol. Chem. **55**: 569, 1917.
TRENNER, S. J. Lab. Clin. Med. **10**: 56, 1924.

THE REACTION OF THE CAT TO ELECTRICAL CURRENTS DIRECTED THROUGH THE HEART

G. H. ETTINGER

From the Department of Medical Research, University of Toronto, and the Department of Physiology, Queen's University, Kingston, Ontario

Received for publication October 1, 1934

The aim of this investigation has been to ascertain the exact effects of low-voltage electrical currents upon the cat, when the currents are conducted through the heart in such a way as to avoid affecting the medulla directly. Careful examinations of the effect of such currents have recently been made by Hooker (1929), Wiggers (1930a, b), and Hooker, Kouwenhoven and Langworthy (1933) upon the dog, and by Ettinger (1933) upon the rabbit. No satisfactory investigation of the effects of electrical currents upon the heart of the cat, has, however, been published.

Maclachlan (1934) has examined 627 authenticated human cases of electric shock from currents of known voltage, after which the patient became unconscious, stopped breathing, and was given artificial respiration. Low voltage shocks, (up to 749 v.) with good contacts, were responsible for 87 victims; 62 (71 per cent) of these were resuscitated. This indicates that the heart of man is highly resistant to such currents, which rarely produce a fatal ventricular fibrillation. The cat was chosen in my experiments as an animal known to resist attempts to produce a fatal ventricular fibrillation, in the hope that some light might be thrown on the reaction of the heart of man to low voltage electrical currents.

The experiments were done with the apparatus and by the method used in experiments upon the rabbit (Ettinger, 1933). Currents varying from 40 m.a. (50 v.) to 1740 m.a. (740 v.) 25 cycle A.C. were applied, on the average, for 3.3 seconds, by leads on the left axilla and the right thigh. The effects were studied by kymographic records of respiration and blood-pressure and by electrocardiograms (lead 2) taken immediately after the current was broken. For the latter most of the animals were curarized, requiring artificial respiration as well. In some experiments the effects were noted by direct observation of the exposed heart.

Forty cats, under very light ether anesthesia, were used. If the first applied current did not cause death, it was reapplied at intervals of 5 to 10 minutes, usually until a fatal ventricular fibrillation was produced. Resuscitation was attempted and was successful on many of the animals.

Currents were again applied to the resuscitated animals. In this way the effects of the same or of varying strengths of current were studied in 201 tests on 40 cats.

The passage of the current threw the heart forward in high tonus, and produced rapid trembling movements which were not expulsive unless the current was below 90 m.a. These movements were not analysed electrocardiographically. After the current was broken either an immediate or a delayed cardiac recovery or a fatal ventricular fibrillation resulted. The exact effect was never predictable, except that currents below 72 m.a. (75 v.) were never fatal.

1. *Immediate recovery.* After the current was broken, coördinate, expulsive beats occurred immediately in 79 of 182 experiments in which the current was of lethal amperage, i.e., at least 72 m.a. (75 v.). These restored the blood-pressure to the normal level, or even higher.

2. *Delayed recovery.* Delayed recovery occurred in 58 of 201 experiments after the application of currents of 20 m.a. (20 v.) to 900 m.a. (400 v.). It succeeded a ventricular incoördination of from 2 to 62 seconds, after which powerful coördinate expulsive ventricular beats occurred which raised the blood-pressure to the normal level or higher.

Electrocardiograms taken in 33 experiments showed an immediate ventricular incoördination without preliminary extrasystoles. Usually the initial deflections were sharp and regular, at the rate of 600 to 700 per minute, gradually slowing to as few as 400 per minute. They were of good voltage, which frequently rose and fell rhythmically. They probably indicated a ventricular flutter. Less often the early deflections were more irregular, resembling the phase described by Lewis as transitional between flutter and fibrillation, but they soon changed to a true ventricular flutter. Terminal gross flutter movements were succeeded by a return to coördination with expulsion. The early expulsive period showed either a few ventricular extra-systoles, or a period of total heart-block with slow idioventricular beats. This was succeeded by a sinus control with a long P.R. interval which gradually became shorter, except when the incoördination had been brief, in which case the conduction was normal as soon as the ventricle followed the auricular lead.

Of the 40 animals used, 25 survived the first application of current. Many of these showed a high resistance to further shocks; 12 survived 5 or more consecutive applications, 5 survived 10 or more, and one animal survived 20 shocks.

3. *Fatal shocks* (fig. 1). When the ventricular incoördination lasted longer than 62 seconds, any return of ventricular coördination (which usually happened), was not accompanied by expulsion and the effect is thus called "fatal." Currents from 72 m.a. (75 v.) to 1740 m.a. (740 v.) (the upper limit of the apparatus) were potentially lethal and actually

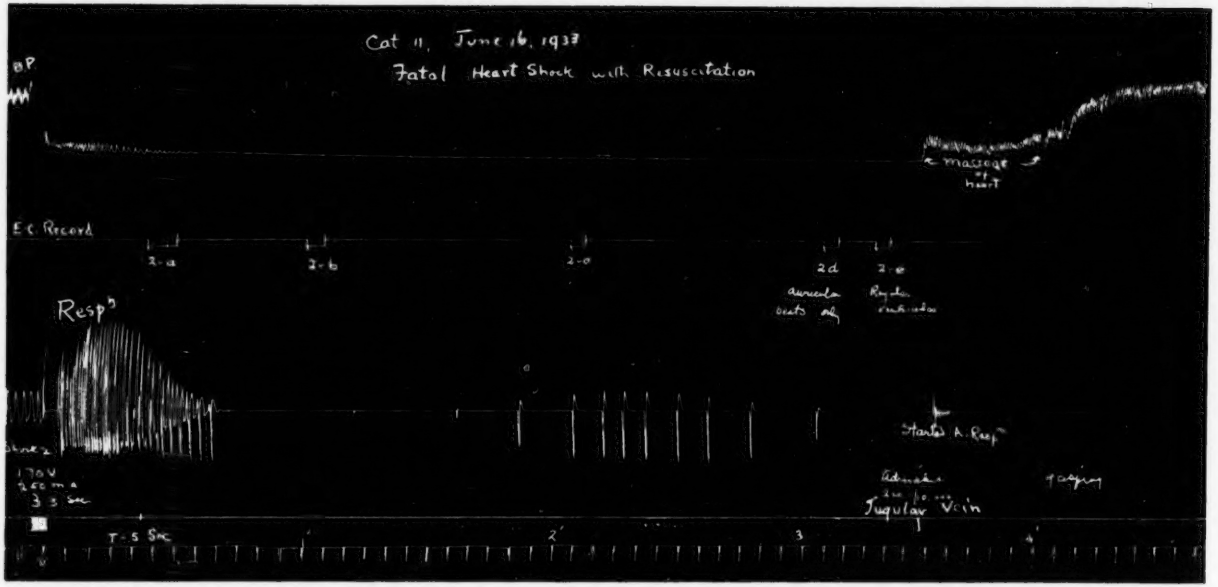


Fig. 1. Fatal shock with resuscitation. Incoördination started at once; the sharp waves on the fallen B.P. record are respiratory effects. The last respiratory gasp occurred 3′ after the shock. Meantime, the electrocardiograph indicated (*2-d* and *-e*) that coördination had returned without expulsion. Adrenaline was given intravenously, the heart massaged and artificial respiration begun; 30″ later expulsion occurred, the circulation was reëstablished and the animal started to gasp.

Electrocardiograms were taken before the shock, during the effects of the shock (*2-a* to *2-e*) and after resuscitation. These are shown in figure 2.

"fatal" in 48 of 182 experiments. Of the survivors of the first application, 6 succumbed to a second, and of the 19 survivors of the first two applications, 4 were killed by a third.

Electrocardiograms were recorded in 43 experiments. There was an immediate ventricular flutter (which might last 40 seconds) or a flutter-fibrillation; either was succeeded in from 27 seconds to 2 minutes by a frank fibrillation. In 35 of the 43 experiments this stopped abruptly in from 1 minute and 40 seconds to 10 minutes and 30 seconds, to be succeeded by absolute ventricular quiet, with a regular auricular beat. (In the exposed heart the auricles were seen to commence to beat within a minute after the electrical circuit was broken.) The auricles beat alone for 2 to 10 seconds, then there were irregular ventricular complexes which shortly became regu-

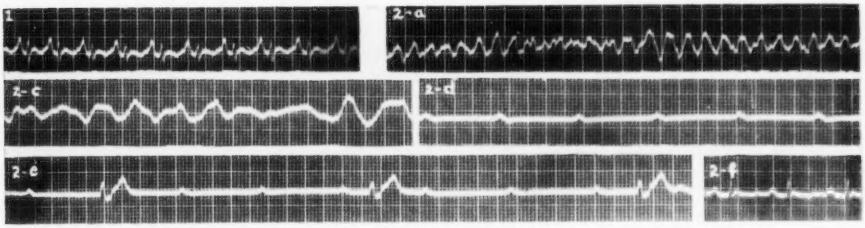


Fig. 2. Cat 11. Fatal shock with resuscitation by adrenaline. See figure 1.

1. Normal record before shock. Lead II: T = 1/10, 1/50 sec.

2-a. Transitional flutter—fibrillation, 25″ after shock.

2-c. True fibrillation, 2′ after shock.

2-d. Sudden ventricular diastole, auricles regular, 3′ after shock.

2-e. Ventricular coördination, total heart-block, inexpulsive beats—3′15″ after shock.

2-f. Normal expulsive beats after resuscitation with adrenaline and massage.

lar but slower than the auricular beats (fig. 2). These idioventricular beats were inexpulsive. If the heart was not stimulated, the auricular beats became slower but registered for as long as 40 minutes. No return of ventricular coördination was seen in only 8 of the 43 experiments, within periods of 13 to 40 minutes. In five of these, however, adrenaline or massage had been administered in an attempt to shorten the fibrillation, and these procedures tended rather to exaggerate it.

The chief distinction, then, between the fatal and non-fatal effects of the electric shock, lies in the length of time of the ventricular incoördination. If this lasts so long that the heart is seriously asphyxiated, or depleted of carbohydrate, the delayed coördinate beat is inexpulsive and the animal perishes. The maximal limit for incoördination with subsequent recovery is just over one minute.

Respiratory changes. As in the rabbit, respiration is prevented during

the passage of the current, but is violent for about two minutes after the circuit is broken. When a fatal effect is produced, regular breathing fails after two minutes, to be followed by a few asphyxial gasps in the third minute.

Resuscitation. Ettinger (1933) found that the coördinate inexpulsive heart-beat which follows ventricular fibrillation in the rabbit could be made expulsive by intravenous injection of adrenaline, accompanied by vigorous cardiac massage. This method was attempted in the cat.

Artificial respiration was instituted or continued after the "fatal shock." When fibrillation had ceased, and it was apparent (within 10 minutes after regular ventricular coördination had been recorded by the electrocardiograph) that expulsive contractions would not be resumed, an injection of adrenaline (usually 2 cc. 1/10,000) was made into the jugular vein, and the heart massaged through the chest. The circulation was restored in 1 minute 30 seconds, on the average, after resuscitation was started. In eighteen attempts on as many cats, there was only one failure. Several animals which were thus resuscitated were "shocked" again, some of them repeatedly. In seven experiments in which the animals were subjected to a second, third or fourth "fatal shock," resuscitation was successful in six attempts; the seventh attempt reprecipitated fibrillation. Thus, the method was effective in 23 of 25 attempts. The total time of medullary anemia, i.e., the interval between the application of the current and the restoration of the circulation varied from three to nineteen minutes. When curare was not used the animals with the shorter periods of circulatory want invariably breathed and exhibited excellent reflexes after resuscitation; one animal with a medullary anemia for 15 minutes was in excellent condition after resuscitation.

Massage of the heart without adrenaline was tried in three experiments without success. The fact that several injections of adrenaline in 2 cc. amounts was necessary in most cases, even though massage was continuous, is additional support for the view that the latter alone is inefficacious.

Massage of the heart alone, before the ventricles had ceased fibrillating, was attempted in five experiments. In all cases it was unsuccessful in stopping the fibrillation, and in four of the five animals no subsequent return to coördination occurred at all. It is remotely possible that massage was not carried on long enough, for Levy (1920), who induced fibrillation with drugs and a faradic current, claims to have had uniform success with this method, although he had to massage in some cases for as long as 48 minutes.

In 7 experiments, in an attempt to stop fibrillation, adrenaline was injected into the ventricles, or into the jugular vein, and the heart massaged vigorously. The fibrillation was exaggerated and prolonged in all, and in five of the seven experiments there was no subsequent return to coördination.

The principles of resuscitation of the heart following electric shock are essentially the same in the cat and dog, namely, the fibrillation must either cease spontaneously, as it usually does quite early in the cat, or it must be stopped, as is usually necessary in the dog, with potassium (Hooker, 1929; Wiggers, 1930), or countershock (Prevost and Batelli, 1899; Hooker, Kouwenhoven and Langworthy, 1933); then expulsive beats must be initiated by the use of calcium or adrenaline with massage; but if the heart has been subjected to asphyxia for too long a period, expulsive beats cannot be initiated.

Cause of the variable response. These experiments indicate that low voltage alternating electrical currents tend to throw the heart of the healthy cat into an inexpulsive ventricular incoördination which is rarely permanent or terminal. The incoördination tends to resolve suddenly into coördination. If this return to coördination is immediate, or delayed no longer than a minute, the heart will manifest expulsive beats which are sufficient to reëstablish the circulation and the animal will recover; if the restoration of coördination is delayed beyond a minute, cardiac asphyxia prevents the return of expulsive beats and the animal dies. In only 8 of 201 experiments did the incoördination fail to cease spontaneously, and five of these had had measures (adrenaline and massage) applied which tend to intensify an established fibrillation. In 41 experiments in which a frank fibrillation had been established for periods beyond a minute, massage not having been attempted within the first 4 minutes, spontaneous coördination failed to recur in only 6, or 15 per cent.

This tendency for coördination to return spontaneously in the cat is not generally reported by other investigators. This may be due to a failure to watch the heart long enough, or to a toxic effect of agents used in producing fibrillation (Levy and Lewis, 1911, 1913). MacWilliam (1889), McGrath and Kennedy (1897), Batelli (1904) and Levy (1911, 1913, 1919) regard such recovery as rare. D'Halluin (1904) finds it common. Garrey (1914) and Wiggers (1930a, b) also record spontaneous recovery in the cat, but provide no data nor an electrocardiographic study. MacWilliam (1918) and Levy (1920) tacitly assume that spontaneous recovery is unlikely when they describe success in terminating fibrillation in the cat, by methods which they apply before and during the period when I have shown spontaneous recovery to be likely to occur.

Since the distinction between fatal and non-fatal effects is based upon the duration of cardiac incoördination, it may be useful to determine what factors affect this period. These will be considered under the following heads: strength of current, mass of heart muscle, excessive ventilation, relation of the incidence of the fibrillation to phases of the cardiac cycle, and depletion of glycogen stores.

1. *Strength of current.* Currents within the potentially lethal range were

"fatal" in only 26 per cent of cases. It was never possible to predict from the strength of the current how long, if at all, incoördination would last after the current was broken. Not only were the effects of the same strength of current variable in different animals, but, when currents of different strength were applied a number of times (as, in some resistant animals, up to 20), to the same animal, there was no direct relation between the strength of the current and the duration of the incoördination, and the same current applied at different times produced disorders of varying duration.

2. *Heart mass.* Garrey (1914) and Hooker (1932) have shown that, in the dog, small pieces cut from a fibrillating ventricle cease fibrillating, and that the tendency of this cardiac muscle to recover from fibrillation, or the difficulty of inducing fibrillation varies inversely as the tissue mass. This raised the question whether the differences in the weight of the hearts might explain the observed variations in response. The following observations indicate that the weight of the heart is not an important factor. Fatal effects were produced in hearts of 5, 9.5, 11.25, 14.25 and 23.5 grams with equal facility, and with great difficulty in other hearts of practically equivalent weights. A 20 gram heart recovered 9 seconds after the first application of current, a 10 gram heart fibrillated for more than 40 minutes. Consecutive equivalent currents applied to the same hearts produced either a prolonged or a transient disorder. Obviously variations in heart mass within the ranges observed in the cat do not explain the variations in response. However, it is not implied that these observations cast doubt on those of Garrey and Hooker.

3. *Excessive ventilation.* Schlapp (1933) has reported that the heart of the artificially ventilated decapitate cat, after several hours, is prone to fibrillation after the injection of adrenaline. The time factor is very important. He believed that this was due in part to overventilation alkalosis, although he found no direct relation between the alkalinity of the blood and the susceptibility of the heart to adrenaline. This suggested the question whether the length of the period of ventilation might explain the variability of the responses of the heart in my experiments.

Most of the animals in this study were curarized while under ether, and given artificial respiration. Prolonged ventricular fibrillation was caused by the first application of current in fifteen of forty animals, and by the first, second or third application in twenty-five animals, i.e., within 30 minutes or less of artificial respiration. Five other cats survived a dozen shocks, given over a period of two to four hours. In some of these, early applications of the current caused disorders lasting just less than a minute while later shocks had little effect. It is obvious that prolonged artificial respiration *per se* was not the cause of the variation in the response of the heart in these experiments.

4. *Relation of the incidence of fibrillation to phases of the cardiac cycle.* De Boer (1921) has shown that it is possible to produce ventricular fibrillation in the frog-heart by a single stimulus applied just at the end of the refractory period. Williams, King, Ferris and Spencer (1934) have shown that, in the sheep, electrical currents applied to the heart for fractions of a second rarely produce ventricular fibrillation unless they are applied during that phase of the cardiac cycle represented by the T-wave of the electrocardiogram. These results raised the question whether the variable response of the cat was due to the phase of the cardiac cycle at the time the circuit was closed.

A rough index of this phase was obtained by running the kymograph quickly and watching the disturbance of the sphygmogram at the time of the shock. The spasm of the skeletal muscles due to the shock always gave a fling to the lever of the Fick type of manometer used in recording blood-pressure. Since this was sharp and well-defined it was accepted as a reliable signal for the application of the current. Such records were obtained in fifty-one experiments on eight cats. It was found that there is no phase of the sphygmogram at which the heart was more liable to go into fibrillation than to recover at once. Indeed the chances of immediate recovery are greater than the chances of a measurable incoördination. Since the T-wave in the cat occupies about one-third of the cardiac cycle, it would have been well covered by the experiments here recorded. However, it should be pointed out that the results of these experiments are not necessarily at variance with those of De Boer and Williams, since in their experiments a single stimulus, or one of short duration (0.03–0.1 second) was employed, and in my experiments the current was of longer duration and covered several cycles.

5. *Depletion of glycogen stores?* It would appear from the following observations that the variation in the duration of the incoördination is due to either an initial sensitivity of the heart or an increase in sensitivity as currents are successively applied, which might be secondary to inadequate storage, or depletion of some protective substance or substances. When the first application of the current did not produce a "fatal" fibrillation, repeated applications, in all but four cases, finally resulted in the production of a fatal fibrillation. Sometimes as many as twenty-one applications of the current were necessary, showing that some of the hearts were much more resistant than others. Moreover, once a "fatal" fibrillation had been produced and the animal resuscitated, succeeding applications of current were prone to induce "fatal" effects regularly. Further, in the cats in which the first application of current caused a "fatal" effect (15 cases), second and third applications of the current also produced "fatal" effects. Although more than one substance may be concerned, glycogen probably deserves prior consideration, because Hooker and Kehar (1933) have

shown that the heart in fibrillation consumes much more carbohydrate than when beating coördinately, and Schenk (1924) has shown that chloroform depletes the glycogen of heart muscle, which may explain in part the ventricular fibrillation which results on the withdrawal of deep chloroform anesthesia in the cat (Levy, 1919).

Although the author has not been able to solve the problem of the cause of the variable duration of the incoördination in the cat, it would appear from the indirect evidence submitted that the cause resides in some cardiac mechanism which in some (15) cats may be initially sub-optimal or rendered sub-optimal (21 cats) by repeated insults. While the size of the heart may help to determine the variation in duration of incoördination between species, according to my observations it does not explain the individual variations in the cat.

CONCLUSIONS

1. A low voltage current directed through the heart of the cat produces an inexpulsive ventricular flutter or transitional flutter-fibrillation which may pass into true fibrillation. In contrast to the usual results in the dog the incoördination usually ceases spontaneously within eleven minutes, but its duration is variable and determines whether or not the electric shock is "fatal." If the incoördination terminates within a minute, the succeeding beats are expulsive and the animal recovers; if it lasts beyond a minute the restored coördination is inexpulsive and the animal dies.

2. When a first or second application of the current is not "fatal" the heart shows a pronounced tendency to prompt recovery from later applications, but may eventually be thrown into a "fatal" fibrillation.

3. The inexpulsive coördinate beat which follows true ventricular fibrillation in the cat may be made expulsive and the animal resuscitated by intravenous injection of adrenaline with vigorous cardiac massage and artificial respiration. As in the rabbit, if the adrenaline is given before the fibrillary process is terminated, fibrillation tends to be exaggerated.

4. A low voltage current (40 to 1740 m.a. at 50 to 740 v.) through the heart is followed by profound respiratory excitement. If the effect is "fatal" the respiratory disturbance may last three minutes, during which time the circulation is completely suspended.

5. Factors which determine the duration of the cardiac disorder produced by an electric shock in the cat are discussed. The results indicate that the duration of the disorder is not dependent on the strength of the current, the mass of heart muscle, the phase of the cardiac cycle at the time the current is applied, nor upon the amount of artificial respiration previously administered. It is suggested that the duration of the disorder may be affected by the glycogen stores of the heart.

Acknowledgment. This research has been sponsored by the Hydro Electric Power Commission of Ontario, under the direction of the Committee on Electric Shock of the University of Toronto. I am deeply indebted to Mr. Wills Maclachlan and Dr. R. E. Gaby, for advice and assistance in procuring special equipment, to Prof. F. G. Banting for providing laboratory facilities, to Prof. C. H. Best for the loan of an electrocardiograph, and to Messrs. F. L. Harrison and J. A. Romeyn for technical assistance.

REFERENCES

BATELLI, F. Richet's Dictionaire de Physiologie **6:** 876, 1904.
DE BOER, S. J. Physiol. **54:** 400, 1921.
D'HALLUIN, M. Presse Med. **12:** 345, 1904.
ETTINGER, G. H. This Journal **105:** 457, 1933.
GARREY, W. E. Ibid. **33:** 397, 1914.
HOOKER, D. R. Ibid. **91:** 305, 1929.
Ibid. **99:** 279, 1932.
HOOKER, D. R., W. B. KOUWENHOVEN AND O. R. LANGWORTHY. Ibid. **103:** 444, 1933.
HOOKER, D. R. AND N. D. KEHAR. Ibid. **105:** 246, 1933.
LEVY, A. G. AND T. LEWIS. Heart **3:** 99, 1911.
J. Physiol **42:** iii, 1911.
Heart **4:** 319, 1913.
Ibid. **7:** 105, 1919.
Ibid. **7:** 175, 1920.
LEWIS, T. The mechanism and graphic registration of the heart beat. Shaw and Sons, London, p. 316, 1920.
MCGRATH, G. B. AND H. KENNEDY. J. Exp. Med. **2:** 13, 1897.
MACLACHLAN, W. J. Ind. Hyg. **16:** 52, 1934.
MACWILLIAM, J. A. Brit. Med. J. **2:** 6, 1899.
Proc. Roy. Soc. **90** B: 302, 1918.
PREVOST, J. L. ET F. BATELLI. J. de Physiol. et de Path. gén. **1:** 427, 1899.
SCHENK, P. Pflüger's Arch. **202:** 315, 1924.
SCHLAPP, W. Quart. J. Exp. Physiol. **23:** 335, 1933.
WIGGERS, C. J. This Journal **92:** 223, 1930a.
Am. Heart J. **5:** 351, 1930b.
WILLIAMS, H. B., B. G. KING, L. P. FERRIS AND P. W. SPENCE. Proc. Soc. Exp. Biol. and Med. **31:** 873, 1934.

FUNCTION OF THE ROUND WINDOW IN HEARING[1]

ELMER CULLER, GLEN FINCH AND EDWARD GIRDEN

From the University of Illinois, Urbana

Received for publication November 19, 1934

Using the technique employed by Wever and Bray (11), Hughson and Crowe have made a comprehensive study of the peripheral auditory mechanism in cats (6, 2, 7, 8). Throughout these experiments the function of the round window was given extended attention. They reported that when the membrane, which closes the round window, "is made rigid by pressing on it with a plug of moist cotton, the perception of spoken words and practically all tones is increased at least 50 per cent" (6). In a later report we learn that "the effect of immobilization of the secondary tympanic membrane with a pledget of cotton previously reported has been repeatedly confirmed. In addition tissue grafts of periosteum placed in the round window niche with sterile precautions grow in that position and when the animals are tested at intervals of from two days to seven weeks transmission upon the operated side is increased from ten to fifty decibels for the different oscillator tones. No other explanation of this phenomenon than immobilization of the secondary tympanic membrane can reasonably be considered at the present time" (8, p. 337).

These arresting conclusions, which depart so widely from orthodox conceptions of round-window function, provided the initial impulse to our investigation. Can an animal whose round-window membranes are thus weighted, damped or immobilized actually *hear better* (respond to fainter sounds) than normally? That is surely a crucial question; to answer it we must have recourse to hearing tests in trained animals.

Test-procedure. The animal (dog) is first conditioned to respond to acoustic stimulation in the usual way. He is placed in a convenient stock with right forepaw resting on a metal grid which can be charged with electricity. The stimulus-tone (1,000 cycles) is sounded for two seconds, directly followed by a charge just strong enough to effect positive retraction of the foot. The dog soon learns to withdraw the paw as soon as the tone begins, thereby escaping the imminent shock. When this condition-

[1] Communication no. 8 from the Animal Hearing Laboratory, established and maintained with aid from the Research Trustees, American Otological Society. Special assistance from the Elizabeth Thompson Science Fund and from the American Academy of Arts and Sciences is gratefully acknowledged.

ing has been thoroughly established, the tone is made progressively weaker; the animal continues to react as before until a level is reached where he begins to falter, responding sometimes but not always. With further reduction of intensity, a point is eventually reached where no response occurs at all; this can only be interpreted to mean that the sound is now completely inaudible to him. By this procedure, standardized in every detail, the animal's normal acoustic threshold is then measured over a period of days or weeks until a stable and consistent performance is attained.[2] It will be seen from table 1, row 3, that the standard error of most of our dogs is less than one decibel; this is a reliability which competent human subjects, as tested in this laboratory, can achieve only with practice and care. When the animal's normal acuity has been satisfactorily measured, he is ready for the critical (post-operative) tests.

Operative procedure. While by no means difficult for a skilled operator, the surgical entry demands careful design and execution if the animal is to be ready for rigorous testing within six hours. *a.* Whatever anesthetic is used should be excreted rapidly enough to permit recovery within this period; we used an ACE mixture. *b.* Cannulation of the trachea below the level of incision, although it increases post-operative depression, is demanded for three reasons. Since the tracheal side of the incision must be strongly retracted to give operator a clear view of fossula rotunda, dangerous interference with breathing results. Complete closure of the air-channel, because of edematous inflammation near the larynx, may occur post-operatively. This edematous condition, even when not fatal, is often attended by a throaty rattle which completely masks the fainter tones and makes accurate hearing-tests impossible. These difficulties are largely obviated by cannulation. *c.* We proceed as follows. Incise skin longitudinally, keeping near to but free of the larynx, for some two or three inches. Clear away fascia just laterad of sterno-hyoid and sterno-thyreoid muscles. Locate the hyoid bone and follow with finger-tip its cornua to their point of attachment in mastoid part of temporal bone. Just mediocaudad of this junction will be felt the smooth, bulging ventral surface of bulla acustica. To make bulla more accessible, the digastric muscle may be retracted sharply laterad. Clear aside connective tissue and vessels; cut away the tough bullar investment, being careful not to rupture the internal maxillary vein and artery. With bone clean and retractors set, ream out a circular opening of 6 to 8 mm., avoiding contact with any internal part of bulla. This opening, if kept well laterad, will in most cases (internal configuration of the middle ear varies markedly in different breeds of dogs) give sufficient view of the bullar cavity for present purposes. The

[2] The methods and precautions developed and used in this Laboratory for securing reliable auditory thresholds are more fully described in reference (4), to which the reader is referred for detailed information.

round-window niche is however distinctly less conspicuous and accessible than in the cat; it is more deeply imbedded in bone, facing more dorso-caudad. Directly opposite the fossula is a bony ledge, which hampers introduction of the gum pledget but on the other hand helps to retain it when once implanted. The membrane itself, lying at base of pit, can seldom be seen through the bulla opening; but the circular rim or edge of the fossule is easily visible and fairly accessible. Now begins the most difficult and critical stage. The plug[3] is gently pressed into the pit; as much as ten minutes may be consumed in so working it into place that the soft mesh-enclosed gum fills completely the fossule from membrane to rim. Very special care was used to prevent any part of it being squeezed over against the stapes.

As soon as the plug fits snugly, the attached threads are led out through the circular opening, which is then closed with a rubber stopper just large enough to fit closely without extending into the bulla-cavity; this was done to exclude extraneous fluids and tissue. Incision was closed with skin clips and animal was left until sufficiently revived for post-operative tests.

A preliminary survey quickly disclosed the approximate limen with plugs IN; this survey was at once followed by several series of tests under standard conditions. The dog was then removed from the stock and the strings gently drawn, with due care not to displace the rubber stopples in the bulla-aperture. Regularly the plugs offered appreciable resistance to dislodgment, indicating that they had been snugly implanted. The whole procedure was simple and brief, requiring no anesthesia and evoking no signs of distress from the animal, save a slight wince just as the plug was being dislodged from the niche. The animal was then returned to the stall and tests with plugs OUT at once resumed. Thresholds with plugs OUT could thus be secured within 15 minutes after the trials with plugs IN; so that it was clearly impossible for the general condition of the animal or for the situation within the ear to change appreciably: and whatever

[3] The plug should be: *a*, plastic, so it will readily assume the irregular conformation of the pit and fit snugly against the membrane; *b*, smooth, so it will not abrade the membrane or lining of the pit; *c*, consistent enough to hold well together, so it can be inserted and removed in one piece; *d*, removable from outside the incision, so that it may be gently withdrawn without re-anesthetizing animal or disturbing middle-ear conditions. Of these requisites, *a* and *b* were best met, of the many substances tried, by ordinary chewing gum; *c*, by fine meshed surgical gauze; and *d*, by threads attached to the gauze. The warmed gum was enclosed in the gauze, the whole constituting an elongated saccule of mesh, filled with the gum-core, to one end of which threads were attached. This gum was used in every case here reported save dog I (b), where a purely cotton-gauze pledget was used instead. Exposure of the gum to live steam or to ordinary sterilizing fluids makes it brittle, so it was not strictly aseptic. This meant that the useful life of a test-animal was about 24 hours after the operation; but this period was found to be sufficient for an adequate series of reliable tests.

TABLE 1

	A	B	C	D	E	F	G	H	I (a)	I (b)	J	K	L (1)	L (2)
1. Dog														
2. Mean Limen	38.7	46.5	43.2	47.2	39.4	46.1	41.6	42.0	53.2		44.4	42.8	42.2	24.5
3. St. Error	1.76	1.09	0.87	0.86	0.32	1.13	0.77	0.71	0.41		1.06	0.84	0.70	0.50
4. No. tests	3	10	10	10	5	5	4	10	10		10	8	9	4
Operation														
5. Bulla stopped	x	x	40.7 42.8	x	x	x	x	x	x	x	36.5	36.7 30.0	42.0	24.0
6. Bulla unstopped			42.8											
7. Plugs IN	7.6 12.4 11.6	21.6 30.4		7.6	14.2	22.9	15.6 18.8	22.4	17.5	16.5	31.0	−2.0	28.0 28.0	16.0 16.0
8. Plugs OUT	12.7	33.0 26.0		13.2	31.0 12.0	32.4	18.8 20.4	31.0	22.0 27.0	20.0	41.5	6.0	38.0 38.0	20.0
9. Gain from remobilizing membranes	1.1	2.6		5.6	16.8	9.5	0.0	8.6	4.5	3.5	10.5	8.0	10.0	4.0

The records of twelve separate dogs, lettered from A to L, are summarized in the table. A few succumbed to the operation before they could be tested; but we wish to emphasize that every animal, without exception, for which round-window tests were secured is here included.

All measurements are stated in decibels. By "mean limen" is meant the normal threshold of the dog as measured directly prior to the operation. The standard error or deviation is computed in the usual way and gives the variability of this mean limen. Row 4 lists the number of individual limens from which the mean limen is computed. Each of these individual limens is derived from not less than two nor more than three full series of trials. A full series in turn extends from an intensity just great enough to evoke a response *every* time (100 per cent) down to a level where the subject *never* responds (0 per cent). In accord with standard pro cedure the limen is defined as that stimulus-level which elicits a response in just 50 per cent of all trials. For example, subject L, plugs IN, at 1,000 cycles, reacted 100 per cent when the tone was attenuated 24 decibels below the standard (zero) level, 50 per cent at 28 db and 0 per cent at 32 db. The limen is therefore 28 db. These limens were computed by Spearman's mean method (10) and differ slightly from the figures given in a preliminary report (3), which were reached by graphical interpolation. In line 5, an *x* signifies that the circular aperture in the bulla was closed with a rubber stopple for all succeeding tests on that animal. In the five cases where figures appear, the animal's hearing was measured at this point to determine what effect (if any) could be ascribed to the surgical entry alone, the round windows being untouched. In two cases (row 6) these stopples were removed and hearing again tested to learn whether their presence or absence might in any wise affect middle-ear function. In section 7 are summarized all tests (except the preliminary trials in finding the approximate locus of the threshold) while the gum plugs were in place. Each figure is the limen derived during a single test-period and may thus incorporate 50 to 100 separate trials. In some cases the post-operative tests were spread over several periods, or were continued under changed conditions; each is then represented by a separate figure. The same statements apply to section 8. In 9 is given the rise of acuity which ensued when the membrane was re-mobilized by withdrawal of the plug. The figure is obtained in every case by deducting the *last* limen with plug IN from the *first* limen with plug OUT. It will be noted that all, with the exception of the 0.0 in G (see comment on individual cases), are positive.

change was likely to occur (e.g., increasing fatigue from continued testing) would oppose rather than favor our conclusions.

Comment on individual cases. It is necessary briefly to analyze the performance of the several animals.

A, B, C, D. These four animals were non-cannulated; because of respiratory noises their mean improvement, upon withdrawal of the plugs, was but small. In A, furthermore, the plugs could not be properly removed, through lack of the strings used with later animals; hence both remained much as before the attempted removal. B showed marked fatigue in the later tests. After prolonged testing (over 60 trials) during the IN series, the first OUT series gave a score of 33.0. During the first half of the next series, the score remained at 32.4, but then fell precipitately to 19.6 in the second half (a mean of 26.0). In view of this fatigue-effect, the tabulated gain of 2.6 seems conservative. C succumbed while the plugs were being introduced; but the previous tests indicate that the operative entry alone (round windows being untouched) had virtually no effect. In first series (40.7), throat noises became so obnoxious that testing had to be discontinued for a time; later the noise decreased, giving a threshold (42.8) virtually identical with the normal average. Removing the rubber stopples also left the figure unchanged at 42.8. While the stopples were thus shown to have no effect, positive or negative, on acuity, they were retained with later animals to protect the ear before and between tests. D had marked respiratory difficulty and was "very sluggish" (protocol) when the OUT tests were reached.

E. Female. With this, the first cannulated animal, the true magnitude of improvement upon remobilizing the membranes began to emerge. Within less than 15 minutes after she had tested 14.2 with plugs IN (44 trials), E tested 31.0 with plugs OUT (56 trials). E was quiet and responsive throughout, so that the change was striking. The score of 12.0 came the following day, when she already showed marked deterioration; she was obviously distressed by the inflamed wound, seemed irritable and restless and panted noisily through the cannula.

F. Female. In good condition before and after withdrawal. Although one string separated from its pledget, which thus remained in situ, a marked gain in acuity appeared.

G. Male. Good condition. After a preliminary score of 15.6, the second under standard conditions was 18.8. With plugs drawn the threshold remained at 18.8. A second series, directly after, gave 20.4, not significantly greater. We were puzzled by this result which seemed to negative our previous observations. Post-mortem examination revealed, however, that both strings had pulled free of their gum-wads, leaving each fossule filled; when lifted out with a probe, each plug retained the precise contour of pit and membrane. Far from being a negative case, therefore, this seems to confirm the other evidence.

H. Male. Vigorous animal; 65 trials before, 55 after withdrawal. Left membrane showed signs of slight lesion.

I. Female. Fair condition. Upon withdrawal acuity rose from 17.5 to 22.0. Protocol records that I became "much noisier" (respiratory blockage) during the first OUT series. Cannula was removed for cleaning and animal at once retested, with threshold of 27.0. She was now "much quieter, about the same as with plugs IN" (protocol). The true gain is therefore probably higher than 4.5. The next day she seemed able to stand a second insertion. A small wad of salinated gauze, no gum, was used but failed to approve itself; it was so loose of texture that it could not be fitted snugly into the niche and tended to work out readily. Animal also showed marked fatigue in the later tests on this day.

J. Male. The first test (windows untouched) showed a loss of 7.9, for reasons unknown to us. In the regular tests, a gain of 10.5 occurred. Animal vigorous and responsive, ears in good condition.

K. Male. Poor condition. With membranes untouched, score is 6 db under normal; with rubber stopples drawn, it falls still more. That this continued decline was due to increasing depression and not to acoustic impairment is shown by the score in the first half (34.0) and in the second half (26.0), the mean being 30.0. The tremendous loss after insertion of the plugs is most unusual. The figure (−2.0) signifies that acuity had fallen below 0.0, our standard (loudest) sound. After withdrawal the figure was 6.0 (50 trials). For some reason this animal was unusually depressed, requiring much care and effort to make any tests at all. Blood clot had formed underneath the one membrane and also filled the pit; the other ear looked about normal.

L. Male. Unusually vigorous. With both bullae opened, round windows untouched, L remained at both 1,000 (col. L1) and 400 (col. L2) cycles within a few tenths of his normal threshold. After insertion of plugs, he tested 16.0 at 400 cycles; and again in a series immediately after, 16.0. Next at 1,000 the threshold was 28.0. L was then removed from the stock and allowed to rest for 15 minutes (the same interval as usually intervened between IN and OUT tests). Upon resumption he again tested 28.0 at 1,000 and 16.0 at 400. His performance was therefore stable and dependable. Fifteen minutes later, plugs OUT, he tested 20.0 at 400, and 38.0 in each of two series at 1,000 cycles. Signs of marked fatigue then began to appear and testing was stopped. In every respect L's performance was ideal; his great vitality kept him alert and responsive throughout the most exacting test-program of any of our animals. After withdrawal he returned within 4 db of his normal score in both frequencies. This animal's testimony was so unequivocal, in our opinion, as to establish the facts beyond any reasonable doubt; we therefore closed the investigation.

Critical evaluation of the data. Several questions may arise in the reader's mind.

1. May the gum-plugs have worked over against the ossicles, and thus have accounted directly for the impaired acuity? While post-mortem inspection failed to reveal, in even a single one of these cases, any gum on or about the ossicles or the oval window, yet the plug *prior* to withdrawal might conceivably have been in contact with them. In addition to our very special care when inserting the plugs we have more direct evidence. It was impossible with our trained animals to cut away the bone and tympanum so as to gain a complete view of the plug *in situ* and thus to see just how it rested with respect to the ossicles. Accordingly we performed the whole operation on an untrained dog, proceeding in every detail as usual. Then we dispatched the animal and broke open each bulla so as to give a full view of plug, ossicles and tympanic cavity in each ear. Both were examined through a wide-field microscope; in neither was there the least trace of gum or gauze anywhere near the stapes or oval window. Both wads rested firmly and snugly within the rim of fossula rotunda. Each was then withdrawn while under observation through the glass. They came out without touching the ossicles, leaving the fossule empty and clean, the membrane unharmed. We therefore answer this question with an assured negative.

2. May not implantation of the plugs have injured the membranes so that they were no longer in normal condition? This did happen once or twice, as above noted, but there is no reason to believe that it happened oftener. To reassure ourselves on this point, we took an untrained specimen and inserted a plug firmly into the pit *seven* times in succession; taking it out after each implantation and examining the membrane most carefully through the microscope to see whether any lesion had occurred. At the end of the seventh trial, the membrane showed no sign of rupture or hemorrhage, but seemed on the contrary indistinguishable from its initial condition. Even if any slight injury did result from inserting the tampons, this injury would affect the tests OUT as much as the tests IN.

3. Why do not all the animals return to their normal level of performance after removal of the plugs? It may be noted that several of the best animals, such as J and L, did come back to their pre-operative thresholds; but it is clear that most of them did not. We were at first puzzled by this phenomenon; but several factors unquestionably contributed to it. 1. Throat-noises. The non-cannulated animals show a mean improvement, after membrane is freed, of 3.10 db., standard error 1.32; the cannulated (not including G, where the gum-plugs remained *in situ* for both IN and OUT tests, nor I (b), where a different style of pledget was used) show a mean gain of 9.70, s.e. 1.40. Most of this improvement was unquestionably due to reduction of associated respiratory noises. Furthermore, the score of I (a) was raised from 22 to 27 db. by simply removing the partially clogged tracheal cannula. Cannulation did not wholly abolish panting or

noisy breathing; but it served to demonstrate the importance of this factor upon the animal's score. 2. With some animals post-operative fatigue was obviously a factor. This was particularly true of the later post-operative tests (OUT), since these were always preceded by a long schedule of tests with plugs IN. Even L displayed definite fatigue by the end. It must be noted that limen-determination, which demands prolonged and intent listening for tones just barely discriminable from silence, imposes a real task on *any* subject whether human or animal; it is hardly surprising therefore that the dogs fatigue more rapidly after a serious operation than they do normally. Fortunately these fatigue effects, if present, operate to reduce the gain and thus make the results more conservative.

DISCUSSION AND CONCLUSIONS. As well known, the standard conception of round-window function has long been that it serves as "Ausweichestelle" and thus facilitates agitation of the cochlear contents by providing a membrane which can oscillate in step with the oval window. This theory has appeared so reasonable and well established that other suggestions have commonly been accepted only as accessory hypotheses to be used in evaluating special or pathological cases. (Cf. Mangold, 1, p. 417.) Runge (5, p. 747) concludes that when as in the normal ear the round window is free, it serves as Ausweichmembran; but when in pathological conditions it becomes covered or blocked (verlegt), then the aqueducts must take over as best they can this function. The doctrine of Hughson and Crowe represents therefore a novel, not to say revolutionary, conception of round window function: that it serves to absorb energy and thus to protect the ear from undue intensities. Nature, in order to protect the ear at high intensities, has by this theory reduced its efficiency at all levels and has raised the limen to all frequencies.

Clinically the picture is obscure. In most cases, when the fossule is filled with fatty or connective tissue, hearing is markedly impaired. Now and then however somebody reports a case of "normal hearing" when the niche is thus filled. Siebenmann mentions an interesting observation. In one case "trat nach mehrmals wiederholter isolierter Tamponade der runden Fensternische ganz regelmässig nicht nur keine Verschlechterung, sondern sogar eine auffallende Besserung der Hörweite für Flüstersprache (von 5 cm. auf 1 m) ein und die untere Tongrenze rückte um zwei Oktaven hinunter" (9, p. 21). In view of the extremely brief notice, it is hard to evaluate this result. It seems to be generally admitted that closure of the niche (blocking of the membrane) does not cause complete deafness. Over against these limited and discordant clinical observations, which are usually complicated by a variety of pathological conditions, the present experiment shows that damping or weighting or partially immobilizing the membrane by a soft tampon which is gently brought into snug apposition with it has at least one effect: *it impairs hearing in every case.*

To what extent these observations accord with the Hopkins results is a question that may well be deferred. But these results cannot fail, in our opinion, to suggest the importance of correlating electrical observations on receptor, nerve or brain with actual performance tests. The two methods, we think, should be used to check and clarify each other at every step. It may not be practicable always to determine whether certain electrical phenomena are confirmed by tests of actual performance; but if each is checked against the other where comparison is possible, we thereby secure some basis for interpolating their relation where it cannot be directly measured. We believe that biologists sometimes fail to recognize the value and reliability of these functional tests in trained animals. The standard errors in our table (row 3) constate what we have repeatedly observed: that the scores of a properly trained dog in these sensory tests are equal in reliability and consistency to those of a competent graduate student and distinctly superior to those of the ordinary clinical patient. Such tests are as objective as any biological measurements can be; and they provide in our opinion the ultimate criterion whether a given phenomenon has sensory significance or not. Until we have definite evidence, e.g., that a given procedure in middle ear or cochlea or eighth nerve is attended by some demonstrable change in a trained animal's response to sound-stimulation, we should be cautious about calling the procedure acoustic in nature or effect.

SUMMARY

In eleven trained dogs, whose normal thresholds of hearing had been accurately measured, the bullae acusticae were opened. Gum-gauze tampons were gently worked into the fossulae rotundae and thus brought into firm apposition with the secondary tympanic membranes. It was found in every case that insertion of these plugs impaired hearing and that withdrawal of them again restored hearing; the mean difference in scores being almost 10 decibels. It is suggested:

a. That damping, weighting or immobilizing the round-window membranes definitely impairs the efficiency of cochlear function.

b. That the *acoustic significance* of a given phenomenon or effect can be demonstrated only by actual tests of hearing; such acoustic value cannot be assumed or inferred merely because that phenomenon or effect has been observed in connection with the middle ear, cochlea or eighth nerve.

REFERENCES

(1) Bethe, A. et al. Handbuch der normalen und pathologischen physiologie, vol. 11. Berlin, 1926.

(2) Crowe, S. J. and W. Hughson. Ztschr. f. Hals- Nasen- u. Ohrenh. **30**: 65, 1931.

(3) CULLER, E., G. FINCH AND E. GIRDEN. Science **78:** 269, 1933.
(4) CULLER, E., G. FINCH, E. GIRDEN AND W. BROGDEN. J. Gen. Psychol. (in press).
(5) HENKE, F. AND O. LUBARSCH. Handbuch der speciellen pathologischen anatomie und histologie, vol. 12. Berlin, 1926.
(6) HUGHSON, W. AND S. J. CROWE. J. A. M. A. **96:** 2027, 1931.
(7) HUGHSON, W. AND S. J. CROWE. Ann. Otol., Rhinol. and Laryngol. **41:** 332, 1932.
(8) HUGHSON, W. AND S. J. CROWE. Acta Oto-laryng. **18:** 291, 1933.
(9) SIEBENMANN, F. Verh. d. deutsch. Otol. Ges., 1899, p. 19.
(10) SPEARMAN, C. Brit. J. Psychol. **2: 227, 1908.**
(11) WEVER, E. G. AND C. W. BRAY. J. Exp. Psychol. **13:** 373, 1930.

A COMPARATIVE STUDY OF THE EFFECT OF TRAUMA ON HEALTHY VIGOROUS DOGS WITH AND WITHOUT ADRENAL GLANDS

W. W. SWINGLE AND W. M. PARKINS[1,2]

From the Biological Laboratory, Princeton University

Received for publication November 23, 1934

Investigators have often commented on the apparent similarity between the signs and symptoms of adrenal insufficiency and those of secondary shock (Sweet, 1918; Rowntree, 1925; Banting and Gairns, 1926; Lucas, 1926; Wyman and tum Suden, 1930; Harrop and Weinstein, 1933; Harrop, 1933; Loeb et al., 1933, and others). Swingle, Pfiffner, Vars, Bott and Parkins (1933) from their own data and those recorded in the literature, listed 31 conditions usually, if not invariably present in adrenal insufficiency, which are also characteristic of secondary shock. The suggestion was made that the symptoms of adrenal insufficiency and of secondary shock were possibly due to one and the same thing, i.e., failure of one of the factors in the blood volume and blood-diluting regulator mechanism—the adrenal cortex. In this communication some of the evidence upon which this suggestion was based is presented.

The type of bilaterally adrenalectomized dog used in the experiments has been adequately described in previous publications from this laboratory (1933 and 1934). The adrenalectomized animals were carefully trained for blood pressure work, but the control, unoperated dogs were usually not subjected to training before use. Dameshek and Loman's (1932) intra-arterial blood pressure method was employed, and the pressures determined in the femoral artery (Parkins, 1934). The level of arterial pressure (taken in conjunction with clinical signs) was the principal criterion used for gauging the degree of shock. The animals were traumatized while under deep nembutal and ether, or morphine, atropine and ether anesthesia and sacrificed at the conclusion of the experiments. Precautions were taken that the animals did not suffer pain. The procedures used to produce traumatic shock were those commonly employed by workers in this field.

[1] Part of the expenses of this investigation were defrayed by a grant from the Josiah Macy Jr. Foundation, New York, and part (later work) by a grant from the Rockefeller Foundation.

[2] Acknowledgments are due Messrs. A. R. Taylor and H. M. Hays for assistance during the experiments.

Comparison of the effect of a single stage bilateral adrenalectomy with unilateral extirpation of one gland, dissection of the remaining adrenal, plus trauma. There seems to be agreement among investigators who have employed the dog that unilateral extirpation of one adrenal is without effect, whereas removal of both glands at a single stage operation is followed by death within a very short interval—anywhere from 8 to 40 hours, and usually within the first 24 hours. Even those investigators most experienced and skillful do not perform the bilateral operation in the dog at a single stage with any expectation of the animal's withstanding the operation and remaining normal for any length of time. Numerous workers have commented on the rapidity with which death follows the complete bilateral operation (single stage) as compared to the two stage extirpation (Strehl and Weiss, 1901; Elliott, 1914; Marshall and Davis, 1916; Rogoff and Stewart, 1926; Banting and Gairns, 1926; Rogoff, 1929, and others).

Rogoff and Stewart (1926) have repeatedly emphasized the necessity for speed and skill in adrenal operations if shock is to be prevented, and have criticized much of the experimental work on the adrenals on the ground that the animals probably died as a result of trauma rather than adrenal insufficiency. However, the criteria employed by them to differentiate between the two syndromes (traumatic shock and adrenal insufficiency) aside from the time element involved, are not clear.

Evidence is presented here indicating that the operative manipulations *per se* necessary in adrenal ablation are relatively unimportant in inducing shock, no matter how much tissue abuse and dissection of the nervous structures associated with the gland are involved. The cause of shock resulting from bilateral extirpation at one sitting is due, in our opinion at any rate, to the complete and sudden loss of all reserve cortical hormone by extirpation of the whole of the adrenal cortex. Eleven animals, seven experimental and four controls, are listed in table 1.

In our hands, bilateral adrenalectomy at one stage in the dog results in death within a short time unless cortical hormone is administered.[3] The shock resulting from the single stage bilateral operation appears despite all precautions. The right adrenal was removed through a right lumbar incision. After closing the wound the animal was turned over and the left adrenal extirpated through a left lumbar incision. Both operations are simple, and practically no bleeding occurs. The total operative time for most of the dogs, including suturing and bandaging was around 30 to 35

[3] The uniform dose of 3 cc. per kilogram was used as the initial intravenous injection for recovery of animals from shock. This dosage (3 cc. per kgm.) was administered on successive days intraperitoneally in divided doses, half in the morning and half in the evening, until the arterial pressure attained the normal level. The animals were then placed on the maintenance dose which was given subcutaneously. The adequate maintenance dose of cortical hormone referred to throughout this study was from 0.1 to 0.2 cc. per kilogram of body weight per day.

minutes for the complete operation. The blood pressure may remain normal for several hours afterwards and then slowly decline to the death level during approximately 24 hours, the animal exhibiting the classical signs and symptoms of secondary shock. Table 1, dogs 0, 1, 2, 3, 4, 5, and 6.

TABLE 1

Surgical trauma

(Single stage bilateral adrenalectomy)

DOG NUMBER	WEIGHT	NORMAL BLOOD PRESSURE	HOURS AFTER OPERATION	BLOOD PRESSURE	SYMPTOMS	HOURS AFTER OPERATION	BLOOD PRESSURE	SYMPTOMS	REMARKS
					Adrenalectomized				
	kgm.			*mm. Hg*			*mm. Hg*		
0	11.2	105	8	40	Shock	10	0	Dead	Animal not injected
1	9.5	103	3	97	Normal	22	51	Shock	Extract injected; recovery normal within 72 hours
2	10.8	94	5	84	Strong; bright	20	37	Collapse shock	Extract intravenously; recovery
3	11.1	98	4	90	Strong; bright	46	48	Shock	Extract intravenously; recovery
4	11.6	96	16	40	Shock	90	94	Normal	Extract intravenously 16 hours after operation
5	7.4	106	1	110	Nembutal anesthesia	10	42	Shock	Extract intravenously; recovery
6	10.0	110	5	95	Nembutal anesthesia	19	38	Collapse	No extract, allowed to die
					Control dummy operation plus trauma				
7	12.6	96	3	90	None	48	96	Normal	No treatment necessary
8	14.0	108	5	122	None	40	118	Normal	No treatment necessary
9	9.1	104	6	98	None	18	105	Normal	No treatment necessary
10	10.0	99	3	100	None	72	105	Normal	No treatment necessary

Morphine atropine and ether anesthesia employed except in dogs 4, 5, and 6.
Following recovery from anesthesia the dogs were allowed water and food *ad libitum*.
Details of dummy control operation are given in the text.

On the other hand, unilateral right adrenal extirpation through a right lumbar incision followed by a left lumbar incision, dissection of the left adrenal free from surrounding tissues, but with its principal blood supply intact, removal of the left kidney and the spleen, traumatization of loops of the ileum by stripping them through the fingers steadily for five or ten minutes, stretching the mesenteries and vigorous pinching and pulling of

the cut muscles, all fail to induce symptoms of shock or significant decline in the blood pressure. Some of the animals were deliberately kept under ether anesthesia for over two hours.

It has been our experience that so long as one adrenal remains suspended merely by its chief venous connection, the animal may be traumatized with impunity, unless extreme measures are used, so far as the induction of shock symptoms are concerned. For example, dogs 7, 8, 9, and 10, table 1, were subjected to far more extensive surgical manipulation, trauma, hemorrhage and anesthetic than were dogs 0, 1, 2, 3, 4, 5, and 6, table 1. Figure 1 is a graphic representation of the essential data on two controls (nos. 7 and 10) and two typical cases of bilateral extirpation (nos. 1 and 6).

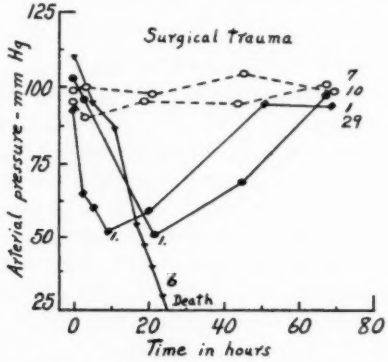


Fig. 1. The effect upon blood pressure, of bilateral adrenalectomy (single stage operation), and unilateral extirpation, dissection of the remaining adrenal plus extensive trauma to adjacent area. Bilateral adrenalectomy, nos. 1 and 6. Dog 6 was not injected; dog 1 was injected with cortical hormone at point marked *1* on the graph. Unilateral operation, plus trauma, nos. 7 and 10. No. 29 shows the effect of a simple operation—ovariectomy—upon the adrenalectomized dog maintained in normal health by cortical hormone. Animal injected with cortical hormone when in shock at point marked *1* on the graph.

The writers have noted that bilateral adrenalectomy at a single stage operation, in the dog, may be followed by hypoglycemia. Significant lowering of the blood glucose seldom occurs if the adrenals are removed at two stages, a week or so apart. We attribute the hypoglycemia which may occur, in part, to the sudden and complete loss of the medullary portion of the glands (Harrop and Weinstein, 1933; Swingle, Pfiffner, Vars and Parkins, 1934). Therefore, in studying the shock following bilateral extirpation of the adrenals in a single stage operation, it is necessary to consider the blood glucose in order to rule out any complicating hypoglycemia as a factor contributing to the observed symptoms.

It is a simple matter to differentiate the shock syndrome following re-

moval of the cortex from the hypoglycemia which may follow the simultaneous removal of the medullary portion of the adrenal in the one stage operation. Intramuscular injections of adrenalin every four to six hours elevate the blood glucose of such animals above the normal level but the shock symptoms due to loss of the cortex remain unaffected. The blood concentrates, the blood chloride and sodium decline, and the arterial pressure steadily diminishes. The animals die presenting the usual shock symptoms despite the normal or elevated blood glucose, or can be revived and restored to normal health by injecting the cortical hormone. These and other data bearing on the alleged relation of the adrenal cortical hormone to carbohydrate metabolism will form the subject matter of a later communication.

The effect of testis trauma on the adrenalectomized dog not receiving extract but free from symptoms, the adrenalectomized dog on a maintenance dose of cortical hormone, the adrenalectomized dog primed with hormone, and the unoperated control. Crushing the testes has been resorted to by various investigators in efforts to induce traumatic shock (Wiggers, 1918; Moore, 1929, and others). In our hands, however, this method has proven quite inadequate to produce symptoms of shock in unoperated control dogs. The animal lacking adrenal glands and temporarily not receiving hormone injections but in normal health, insofar as appearance, activity, appetite, weight, etc., would indicate, does, however, react promptly to testis trauma by developing profound shock. Such animals do not show any tendency toward recovery from this trauma unless injected with cortical hormone, whereupon the shock symptoms rapidly disappear.

The testes were crushed in a vise by slowly increasing pressure while the animals were under anesthesia. The essential data are given in figure 2 and table 2. It is evident that dogs without adrenal glands or injected cortical hormone, but apparently normal otherwise except for some decline in blood-pressure, differ remarkably from dogs with intact glands in response to testicular trauma. Such traumatized animals lacking adrenals die within 24 hours or less (usually less) presenting all the typical symptoms of secondary shock unless treated with adrenal cortical hormone. Several animals were deliberately sacrificed by withholding extract following trauma in order to test this point. It is of interest to note that control, unoperated dog 18, figure 2, table 2, was subjected to both hemorrhage (35 cc. per kgm.) and trauma, without signs of shock appearing. The fall and rise in mean arterial pressure as indicated on the graph for this animal are merely temporary responses to the hemorrhage.

The experiment in which the animal was primed with cortical hormone previous to trauma is of especial interest. For example, dog 12, table 2, figure 2 was given an intravenous injection of 20 cc. of extract the night previous to use in the experiment, and received an additional 10 cc. a few

minutes before the testes were traumatized. This dog did not develop any signs of shock but remained normal throughout the experiment. It seems clear that by priming the animal with hormone shock can be prevented.

The effect of testis trauma was tested on one dog which had been bilaterally adrenalectomized 11 months previously and maintained in normal

TABLE 2

Testis trauma in normal and adrenalectomized dogs

DOG NUMBER	WEIGHT	NORMAL BLOOD PRESSURE	DAYS OFF EXTRACT	BLOOD PRESSURE AT BEGINNING OF TRAUMA	BLOOD PRESSURE AT END OF TRAUMA	HOURS AFTER TRAUMA	BLOOD PRESSURE AT TIME OF EXTRACT INJECTION	SYMPTOMS	BLOOD PRESSURE 48 HOURS LATER	SYMPTOMS
				Adrenalectomized						
	kgm.									
11	10.0	93	1	80	59	8	52	Shock	100	Normal
12	11.4	97	0*	98	102	5	105	Normal	102	Normal
13	10.4	106	3	85	54	8	46	Shock	112	Normal
14	13.6	103	1	94	86	30	78	Mild shock	96	Normal
15	11.4	99	2	84	50	9	46	Shock	—Discontinued; pneumonia	
16	12.2	96	—**	100	73	4	60	Mild shock	94†	Normal
				Normal						
17	11.8	110	—	114§	105	4	113	Normal	108	Normal
18	12.7	132	—	93‡	56	4	91	Normal	114	Normal
19	12.8	104	—	112§	92	4	109	Normal	101	Normal
20	10.0	120	—	156§	156	4	126	Normal	116	Normal
21	13.6	120	—	144§	129	4	126	Normal	115	Normal
22	12.7	109	—	150§	139	4	115	Normal	112	Normal

* Primed with 30 cc. cortical extract previous to trauma.

** Dog on minimum maintenance dose of extract.

† Eighty-eight hour recovery.

‡ Pressure 50 minutes after withdrawal of 35 cc. of blood per kilo. Untrained.

§ Under ether anesthesia. Untrained for blood-pressure determinations.

health by daily injections of cortical hormone. The dog weighed 12.2 kgm. and at the time he was traumatized was receiving a total daily dose of hormone of 1.2 cc. He reacted to testis trauma in a manner different from the adrenalectomized dogs not receiving their usual daily dose of hormone on the day they were traumatized. Table 2, dog 16. The animal developed mild shock (Bp at 60 mm. Hg) but slowly recovered without the necessity of increasing the hormone dosage. The recovery process was slow and

difficult however. Evidently the amount of hormone he received from the daily injections was barely sufficient to pull the animal through the crisis precipitated by trauma.

The effect of intestinal manipulation on the adrenalectomized dog maintained in normal health by maintenance doses cortical hormone, and the unoperated control. Manipulation of the intestines has been widely employed as a method of inducing traumatic shock (Burge and Neill, 1917–18; Wiggers, 1917–18; Mann, 1919–20; Erlanger, Gesell and Gasser, 1919; Gasser, Erlanger and Meek, 1919–20; Blalock, 1931; Roome, Keith and Phemister, 1933; Henderson, 1910–11; Guthrie, 1917, and others). It requires pro-

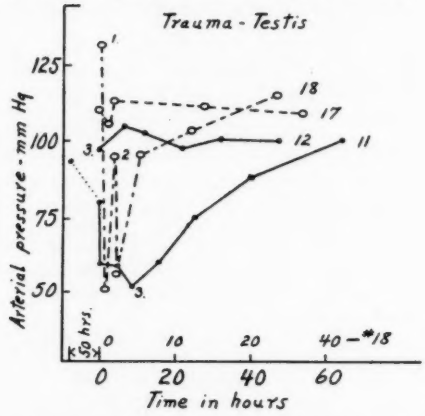


Fig. 2. The effect of testis trauma upon the arterial pressure of the adrenalectomized dog off extract but free from symptoms; the adrenalectomized animal primed with cortical hormone previous to trauma, and the unoperated control. No. 11 adrenalectomized, 50 hours off extract; *3* = point when cortical hormone injected, no. 12 = adrenalectomized but primed with cortical hormone, no. 17 = control, no. 18 = control subjected to hemorrhage, 35 cc. per kilogram at *1* and testis trauma at *2* on the graph. Note that the time in hours is plotted above the base line for dog 18 and below the line for the remainder of the animals.

longed exposure and extensive manipulation of the intestines to reduce the mean arterial pressure to the shock level in the normal dog. On the other hand a negligible amount of handling and exposure of the intestines of the adrenalectomized animal kept in normal health by maintenance doses of cortical hormone, is sufficient to produce fatal shock.

Figure 3, table 3, dog 23 shows that 10 minutes of gentle stripping of the intestine will throw the animal into shock. Experiments performed on control unoperated dogs with intact adrenals reveals that this degree of intestinal trauma is quite negligible and without demonstrable effect upon the animals. Indeed, as shown in table 3, dogs 25 and 28, one or even two

and one-half hours of vigorous stripping of the entire length of the small intestine between the fingers does not induce shock, nor significant lowering of the arterial pressure. Moreover hemorrhage of 35 cc. per kgm. plus thirty minutes of intestinal stripping, is without effect. Table 3, dog 26, figure 3.

In our experience it requires hours of exposure and continuous manipulation of the intestine to reduce the blood pressure to the shock level, i.e., to

TABLE 3

Trauma (various types) in normal and adrenalectomized dogs

DOG NUMBER	WEIGHT	NORMAL BLOOD PRESSURE	HOURS AFTER TRAUMA	BLOOD PRESSURE	SYMPTOMS	HOURS AFTER TRAUMA	BLOOD PRESSURE	SYMPTOMS	REMARKS
				Adrenalectomized					
	kgm.								
23	11.4	100	13	48*	Shock	70	99	Normal	Intestine stripped 10 minutes
24	14.9	96	1–	36	Shock	3	0	Death	Intestine stripped 30 minutes
29	11.7	98	9	52*	Shock	42	95	Normal	Ovariectomy
30	15.3	96	17	62*	Shock	45	96	Normal	Ovariectomy
				Normal					
25	10.0	105	4	109	Normal	45	113	Normal	Intestine stripped 1 hour
26	11.1	110	5	106	Normal	36	98	Normal	Hemorrhage** and trauma
27	10.0	108	1	82	Normal	3–19	96	Normal	Hemorrhage† and trauma
28	9.1	116	1	120	Normal	5	130	Normal	Intestine stripped 2½ hours

* Cortical hormone injected at this pressure level.

** Thirty-five cubic centimeters per kgm. and intestine stripped 30 minutes.

† Twenty-five cubic centimeters per kgm. intestines stripped 15 minutes.

40 or 50 mm. Hg in dogs with adrenal glands intact. In such cases the entire intestinal tract has been so severely injured that the animals are unable to hold water without violent vomiting, and it is unreasonable to attempt their resuscitation. However, the slight manipulation sufficient to produce profound shock in the adrenalectomized dog is without structural impairment to the intestinal tract. Injection of adequate amounts of cortical hormone restores such animals to normal. Figure 3 is a graphic representation of these experiments.

Effect of a simple operation—ovariectomy—on the adrenalectomized dog maintained in normal health by maintenance doses of cortical hormone. It was considered worth while to test the effect of a simple operative procedure on the adrenalectomized dog maintained in normal health by maintenance doses of hormone. Numerous experiments had demonstrated the fact that the anesthetic alone is without significant effect upon the blood pressure of these animals. Since ovariectomy is a simple procedure, and swiftly and easily performed, two animals were subjected to the operation. Table 3, dogs 29 and 30. Needless to state, this operation is without effect upon the blood pressure of the normal dog with intact adrenals. Table

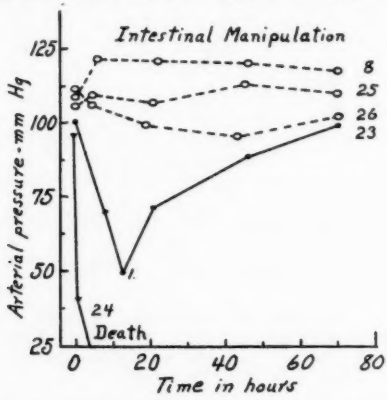


Fig. 3. The effect of intestinal manipulation (stripping) upon the mean arterial pressure of the adrenalectomized dog maintained in normal health by cortical hormone, and the control. No. 24 adrenalectomized 9 months previous, intestine stripped 30 minutes not injected with hormone; no. 23 adrenalectomized 10 months previous. Intestine stripped 10 minutes. *1* = cortical hormone injected intravenously. No. 25 control, intestine stripped 1 hour, no. 8 control., right adrenal and kidney extirpated, dummy operation of left adrenal, left kidney traumatized spleen removed and intestine stripped 10 minutes. No. 26 control, hemorrhage 35 cc. per kilogram plus 30 minutes of intestinal stripping.

3 gives the essential data, and shows that shock follows this operation in the dog lacking adrenals but otherwise in perfect health. The animals were restored to normal health by injections of cortical hormone. Considerable accumulation of fluid within the peritoneal cavity and copious "weeping" of the serous membranes was noted as the operation was in progress. Each ovariectomy including sewing and bandaging was completed within 15 minutes. Practically no bleeding occurred. Essential data on dog 29 are given in figure 1 and table 3.

The effect of muscle trauma on the adrenalectomized dog maintained in normal health, and the control unoperated animal. Trauma to muscle

masses and bones has been extensively employed by students of experimental shock. (Bayliss and Cannon, 1919; Cannon, 1919; Aub, 1920–21; Smith, 1927–28; Moore, 1929; Blalock, 1930; Parsons and Phemister, 1930; Johnson and Blalock, 1931; Roome, Keith and Phemister, 1933; Freed, 1933, and others.) This type of trauma, even in slight degree, induces profound and fatal shock in the adrenalectomized dog maintained in normal health and vigor by maintenance doses of cortical hormone. The unoperated dog with intact adrenals requires a far greater degree of trauma to produce a significant decline in arterial pressure than does the equally vigorous and healthy adrenalectomized animal maintained in normal condition by cortical hormone. The difference between the two types of animal in this respect is surprising, to say the least.

TABLE 4

*Muscle trauma in adrenalectomized dogs**

DOG NUMBER	WEIGHT	NORMAL BLOOD PRESSURE	HOURS AFTER TRAUMA	BLOOD PRESSURE	SYMPTOMS	HOURS AFTER HORMONE INJECTION	BLOOD PRESSURE	SYMPTOMS
	kgm.							
31	12.8	98	4	26–0	Death**			
32	13.0	110	11	51	Shock	36	100	Normal†
33	11.3	108	6	47	Shock	18	94	Normal
34	10.0	109	10	42	Shock	36	103	Normal
35	12.8	111	5	0	Death**			
37	10.9	103	12	51	Shock	36	98	Normal

* Trauma of the degree used in these experiments has negligible effect on the normal animal with intact adrenals. Complete data on large number unoperated dogs will be presented elsewhere.

** Animal not injected with cortical hormone.

† Animals eating full rations.

The animals were deeply anesthetized, and the upper half of the hind limb placed on an iron block and traumatized by blows from an iron club one inch in diameter. Care was taken that the skin remained unbroken and that the bones were not fractured. It is difficult to quantitate the degree of trauma by this method so that each animal receives a comparable amount of tissue abuse. However, efforts were made to traumatize by a definite number of blows of similar strength per kilogram of body weight. The pertinent data are given in table 4 and figure 4.

During the course of the experiments a point arose which, so far as the writers are aware, has either escaped attention or has not been sufficiently emphasized by investigators of traumatic shock. The unoperated dog, kept under anesthesia throughout the experiment, almost invariably succumbs to a lesser degree of trauma than he does if permitted to revive

from the anesthetic following trauma, to the point where he freely drinks water. For example, a dog with a resting arterial pressure of 104 mm. Hg (under nembutal, ether, or other anesthetic) passes into shock with a blood pressure of 40 mm. Hg following a certain degree of trauma. If the animal is not permitted access to fluid, death ensues, or if the anesthetic is removed but no fluid allowed, the arterial pressure remains low, the shock symptoms persist, and the animal dies. However, if following trauma and a blood pressure at 40 mm. Hg, the dog is permitted to drink water, he eagerly ingests large quantities, the blood pressure rises and may return to the normal level within five or six hours and remain so indefinitely. Thus,

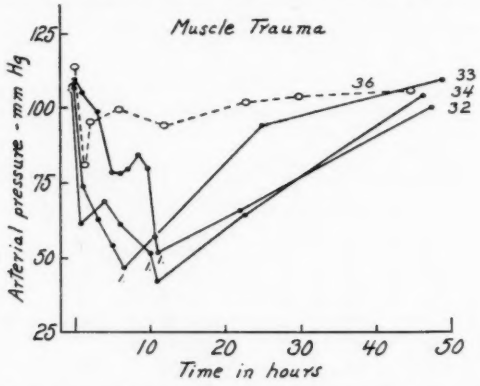


Fig. 4. The effect of muscle trauma upon the mean arterial pressure of the adrenalectomized and unoperated dog. Nos. 32, 33 and 34 adrenalectomized. Mild trauma to one leg. Injected intravenously with cortical hormone at point marked *1* on graph. Animals in profound shock with a falling blood pressure at time of injection. No. 36 unoperated control. Severe trauma to both hind limbs. Two adrenalectomized dogs 31 and 35 (table 4) were allowed to die of shock by withholding cortical hormone. All animals were bilaterally adrenalectomized 2 to 5 months previous to use in these experiments.

it is quite useless to attempt to restore the dog to normal in the absence of adequate fluid intake. If such fluid intake is allowed, a large percentage of the animals, which under the anesthetic and without fluid would die, spontaneously recover. It would seem that traumatizing an anesthetized animal until the arterial pressure falls to 40 mm. of Hg and remains there, and then attempting to restore the dog to normal by various procedures is foredoomed to failure unless provision is made for restoration of the greatly depleted volume of circulating fluid, lost by transudation into the injured area. This can be accomplished by 1, allowing adequate fluid intake, and 2, assuring the presence of a factor which normally serves to hold the fluid in circulation after it is ingested. This latter factor the writers believe to be the adrenal cortical hormone.

In this connection it is interesting to note that following trauma the dog lacking adrenals is unable to utilize the ingested water. Hundreds of cubic centimeters may be taken but in the absence of the adrenal cortex or of sufficient cortical hormone the fluid is not held in circulation, the arterial pressure fails to rise and death invariably ensues. Injections of adequate amounts of cortical hormone restore these profoundly shocked animals to normal health and vigor. The essential data are given in table 4 and figure 4.

The fact mentioned above regarding water intake in the shocked unoperated dog holds true for the intact animal prostrate from hemorrhagic shock. If the unanesthetized animal is bled until the arterial pressure falls to 40 mm. Hg and remains stabilized at this point, and the dog is not permitted to drink water, death results. But if he is allowed free access to fluid he drinks eagerly and rebuilds his fluid volume with concomitant restoration of the blood pressure and complete recovery (Swingle, Pfiffner, Vars and Parkins, 1934).

Discussion. There are profound differences with regard to susceptibility to the effects of trauma, between control unoperated dogs and adrenalectomized animals maintained in normal health and vigor by adequate daily injections of cortical hormone. The control dog with intact adrenals requires severe trauma, involving extensive crushing of muscles and often bones, or prolonged (hours of continuous stripping of the intestines between the fingers) intestinal manipulation to reduce the mean arterial pressure to a shock level from which it will not rise spontaneously. On the other hand, the adrenalectomized dog of similar size, with well healed wounds, free from infection or symptoms of any kind, vigorous, active, eating full rations and at peak weight, rapidly develops fatal shock following a degree of trauma so slight that it would be quite negligible to a dog with adrenal glands. (See also Freed, 1933—Adrenalectomized rats.)

It is interesting to note in this connection that the Addison's disease patient is a notoriously poor surgical risk and rarely if ever survives even minor operations and may pass into fatal shock from merely having a tooth extracted. It would seem, however, that if properly primed with cortical hormone beforehand such patients should be able to withstand major surgical procedures.

The data obtained from study of this series of animals clearly indicate that the unoperated dog has sufficient reserve hormone in his intact functioning adrenal cortical tissue to prevent shock following hemorrhage and trauma, unless very severe measures are employed. The adrenalectomized animal enjoying equally good health but lacking such reserves, passes into fatal shock as a result of trivial injury. The presence or absence of the cortical hormone appears to be the only variable involved in the two types of experimental animal. Further evidence for this conclusion is furnished

by the experiment where the adrenalectomized dog was primed with hormone previous to traumatizing the testes (dog 12, fig. 2) as a result of which no symptoms of shock were produced. However, by far the most convincing evidence showing the relation between the adrenal cortical hormone and traumatic shock, is the fact, amply demonstrated in the experimental data, that injections of cortical hormone restore to normal health and vigor, animals which would otherwise succumb within a few hours to traumatic shock. Studies of the relation of the adrenal cortical hormone to traumatic shock in the unoperated dog, and the accompanying fluid shift and electrolyte changes in shock, are now in progress in this laboratory. Suffice it to state here that the evidence so far obtained indicates that the adrenal cortical hormone plays an important rôle in the prevention of, and recovery from, traumatic shock in normal unoperated dogs.

The reason for the failure of bilateral adrenalectomy at a single stage operation in the dog seems clear. Sudden deprivation of all adrenal tissue rapidly leads to development of two syndromes—shock due to loss of the cortex, and usually, hypoglycemia owing to loss of the medulla. These two syndromes can be distinguished and treated separately, the hypoglycemia yielding to intramuscular injections of adrenalin without influencing the shock, and the shock yielding to injections of cortical hormone.

SUMMARY AND CONCLUSION

1. A comparative study was made of the effect of various types of trauma (surgical, testis, intestinal, and muscle) on the arterial pressure and clinical condition of healthy, vigorous dogs with and without adrenal glands.

2. The type of adrenalectomized animal employed had well healed wounds, was free from infection or symptoms of any kind, and was vigorous, active, eating full rations, and at peak weight.

3. Such animals are extremely susceptible to trauma of any sort and rapidly develop profound and fatal shock following a degree of trauma so slight that it is quite negligible to a dog with adrenal glands.

4. The dog with adrenal glands requires far more severe trauma, involving extensive crushing of muscles and often bones, or prolonged intestinal manipulation to reduce the mean arterial pressure to a shock level from which it will not rise spontaneously.

5. The data indicate that the unoperated dog has sufficient reserve hormone in his intact functioning adrenal cortical tissue to prevent shock following hemorrhage and trauma (unless extreme measures are undertaken to override the glands; data on this point to be presented later). The adrenalectomized animal enjoying good health but lacking such reserve hormone, succumbs to shock as a result of trivial injury.

6. Further evidence for this conclusion (no. 5) is furnished: 1, by priming the adrenalectomized dog with hormone previous to testis trauma, as a result of which the blood pressure remained unchanged and symptoms of

shock did not appear; 2, restoration of the animal from profound shock and collapse to normal health and vigor by intravenous injections of cortical hormone.

REFERENCES

(1) Aub, J. C. This Journal **44:** 388, 1920–21.
(2) Banting, F. G. and S. Gairns. This Journal **77:** 100, 1926.
(3) Bayliss, W. M. and W. B. Cannon. Report of Shock Committee, British Medical Research Committee, no. 25, 1919.
(4) Bayliss, W. M. Report British Medical Research Committee, no. 26, 23, 1919.
(5) Beard, J. J. and A. Blalock. Arch. Surg. **22:** 617, 1931.
(6) Beard, J. W. and A. Blalock. Arch. Surg. **22:** 617, 1932.
(7) Blalock, A. Arch. Surg. **22:** 314, 1931.
(8) Blalock, A. Arch. Surg. **20:** 959, 1930.
(9) Burge, W. E. and J. Neill. This Journal **45:** 286, 1917–18.
(10) Cannon, W. B. Special Report British Med. Res. Comm. no. 26, 27, 1919.
(11) Cannon, W. B. Traumatic shock. D. Appleton & Co., New York, 1923.
(12) Dameshek, W. and J. Loman. This Journal **101:** 140, 1932.
(13) Elliott, T. R. J. Physiol. **49:** 38, 1914.
(14) Erlanger, J., R. Gesell and H. S. Gasser. This Journal **49:** 90, 1919.
(15) Freed, S. C. Proc. Soc. Exp. Biol. and Med. **30:** 677, 1933.
(16) Gasser, H. S., J. Erlanger and W. J. Meek. This Journal **50:** 31, 1919–20.
(17) Guthrie, C. C. J. A. M. A. **69:** 1394, 1917.
(18) Harrop, G. A. Ann. Int. Med. **6:** 1579, 1933.
(19) Harrop, G. A. and A. Weinstein. J. Exp. Med. **57:** 305, 1933.
(20) Henderson, Y. This Journal **28:** 152, 1910–11.
(21) Johnson, G. S. and A. Blalock. Arch. Surg. **23:** 855. 1931.
(22) Loeb, R. F., D. W. Atchley, E. M. Benedict and J. Leland. J. Exp. Med. **57:** 775, 1933.
(23) Lucas, G. H. W This Journal **77:** 114, 1926.
(24) Mann, F. C. This Journal **50:** 86, 1919–20.
(25) Marshall, E. K. and D. M. Davis. J. Pharmacol. Exp. Therap. **8:** 525, 1916.
(26) Moore, R. M. This Journal **89:** 508, 1929.
(27) Parkins, W. M. This Journal **107:** 518, 1934.
(28) Parsons, E. and D. B. Phemister. Surg. Gynec. and Obst. **51:** 196, 1930.
(29) Rogoff, J. M. Endokrinol. **5:** 256, 1929.
(30) Rogoff, J. M. and G. N. Stewart. This Journal **78:** 683, 1926.
(31) Roome, N. W., W. S. Keith and D. B. Phemister. Surg. Gynec. and Obst. **56:** 161, 1933.
(32) Rowntree, L. G. J. A. M. A. **84:** 327, 1925.
(33) Smith, M. I. J. Pharmacol. Exp. Therap. **32:** 465, 1927–28.
(34) Strehl, H. and O. Weiss. Pflüger's Arch. **85:** 107, 1901.
(35) Sweet, J. Am. J. Med. Sci. **155:** 627, 1918.
(36) Swingle, W. W., J. J. Pfiffner, H. M. Vars and W. M. Parkins. This Journal **107:** 259, 1934.
(37) Swingle, W. W., J. J. Pfiffner, H. M. Vars, P. A. Bott and W. M. Parkins. Science **77:** 58, 1933.
(38) Van Slyke, D. D. J. Biol. Chem. **83:** 449, 1929.
(39) Wiggers, C. J. This Journal **45:** 435, 1917–18.
(40) Wiggers, C. J. This Journal **46:** 314, 1918.
(41) Wyman, L. C. and C. tum Suden. This Journal **94:** 579, 1930.

THE MECHANISM OF THE CIRCULATORY CHANGES ACCOMPANYING INSULIN HYPOGLYCEMIA

A. CARLTON ERNSTENE, JOSEPH E. F. RISEMAN, BEATRICE STERN AND BENJAMIN ALEXANDER

From the Medical Research Laboratories, Beth Israel Hospital, and the Department of Medicine, Harvard Medical School

Received for publication November 26, 1934

Insulin hypoglycemia in man usually is attended by an increase in the minute volume of the heart and in the ventricular rate (Ernstene and Altschule, 1931). Cannon, McIver and Bliss (1924) demonstrated that in cats whose hearts had been denervated completely and in which one suprarenal gland had been removed and the other denervated, the heart rate did not increase during hypoglycemia as it did in animals with denervated hearts but with intact suprarenal glands. They concluded that the increase in heart rate in the latter animals resulted from a discharge of adrenin in response to stimulation of the sympathetic nerves to the suprarenal glands. Ernstene and Altschule suggested that a similar mechanism might be responsible for the increase in cardiac minute volume output observed in man during hypoglycemia. The present investigation was undertaken in order to test the validity of this hypothesis.

METHODS OF STUDY. Cats weighing 1.1 to 4.1 kgm. were used throughout the investigation. Three groups of animals were studied: 1, normal animals; 2, animals in which the heart had been denervated according to the technic of Cannon, Lewis and Britton (1926), and 3, animals with denervated hearts in which the suprarenal glands had been inactivated by removing the right suprarenal and denervating the left. All operative procedures were carried out under ether anesthesia, and the animals usually were allowed to convalesce for 10 to 30 days before the actual experiments were made.

Food was withheld for 16 hours before all experiments but the animal was allowed to have water. Anesthesia was induced by the intraperitoneal administration of amytal in 1 per cent solution. Throughout the experiment the rectal temperature was controlled by means of a heated animal board, so that the maximum variation was not more than 1.0°F. The expired air from the animal was collected in a calibrated spirometer by means of a tracheal cannula connected to a set of small Krogh valves.

When the heart rate, respiratory rate and rectal temperature had

reached a constant level and the air-collecting system had been completely washed not less than six times, the expired air from the animal was collected in the spirometer for an accurately measured period ranging from four to eight minutes in different experiments. Samples of arterial and mixed venous blood then were obtained by puncture of the right and left ventricles; the two samples amounted to 2 cc. each and were obtained as nearly simultaneously as possible, usually within a few seconds. Duplicate measurements of the oxygen content of each sample were made according to the micromethod of Van Slyke and Neill (1924). A small fraction of one specimen was used for measurements of the blood sugar content according to the micromethod of Folin (1928, 1929). Samples of the expired air and of the room air were analyzed for their content of oxygen and carbon dioxide. The results of these analyses and the corrected respiratory minute volume were utilized to determine the amount of oxygen consumed per minute by the animal (Boothby and Sandiford, 1920). The minute volume output of the heart was calculated from these data according to the formula of Fick (1870).

The cardiac punctures caused a temporary rise in heart rate amounting usually to less than 20 beats per minute. In a few instances, however, an increase of more than 25 beats per minute was noted. The rate generally returned to within 10 beats of the original level within 30 minutes, and at this time several of the cats in each group were given approximately 4 units of insulin per kilogram of body weight by intramuscular injection. Observations on the heart rate, respiratory rate and temperature were continued at intervals of 5 minutes for $1\frac{1}{4}$ to $2\frac{1}{4}$ hours. The procedures necessary for calculation of the minute volume output of the heart were then repeated.

In a second type of experiment the animals received no insulin between the two measurements of the cardiac minute volume output. These experiments served as control observations.

Results. Observations were made before and during insulin hypoglycemia in nine cats with denervated hearts, in nine with denervated hearts and inactivated suprarenal glands, and in seven normal animals. The initial sugar content of the blood in these cats varied between 85 and 133 mgm. per 100 cc. and the content at the end of the experiments ranged from 23 to 78 mgm. per cent. The results of typical experiments are presented in figures 1 and 2, and the average values for the percentage change in heart rate and cardiac minute volume output in each group of animals are recorded in figure 3.

Control experiments were made on three cats with denervated hearts, on two with denervated hearts and inactivated suprarenal glands, and on seven normal animals.

Before presenting the results pertaining to changes in the minute volume

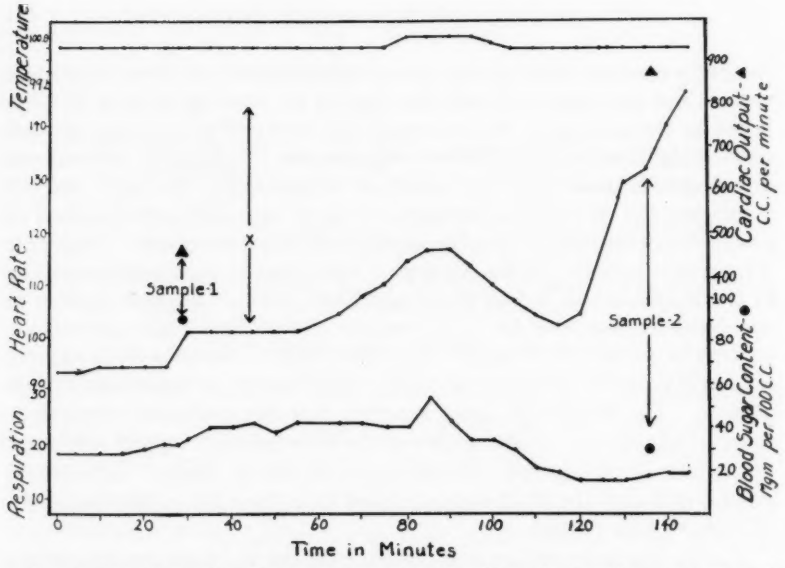


Fig. 1. The effect of insulin hypoglycemia on cardiac minute volume output and ventricular rate in cats with denervated hearts and intact adrenal glands.

Summary of a representative experiment. Cat 20. Weight 2.81 kgm. Eleven units of insulin injected intramuscularly at x.

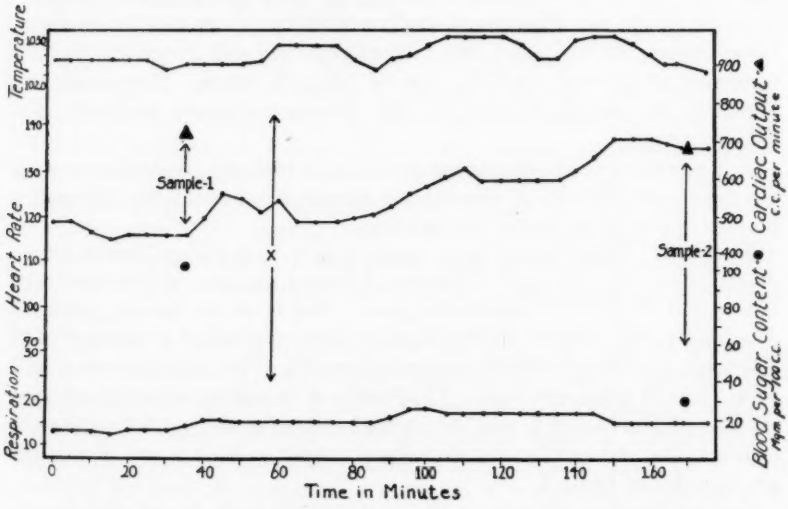


Fig. 2. The effect of insulin hypoglycemia on cardiac minute volume output and ventricular rate in cats with denervated hearts and inactivated adrenal glands.

Summary of a representative experiment. Cat 31. Weight 2.93 kgm. Twelve units of insulin injected intramuscularly at x.

output of the heart, we shall review briefly the changes in heart rate and the component data upon which the calculations of the cardiac minute volume output were based.

The effect of insulin hypoglycemia on heart rate. Hypoglycemia was attended by an increased heart rate in all animals with denervated hearts and intact suprarenal glands. In seven of the nine cats, the increase amounted to more than twenty beats per minute, and the average rise for the entire group was thirty-four beats per minute (26 per cent). In contrast, the heart rate remained unchanged or decreased during hypoglycemia in seven of the nine cats with denervated hearts and inactivated suprarenal glands. In the other two, an increase of twelve and eighteen beats per

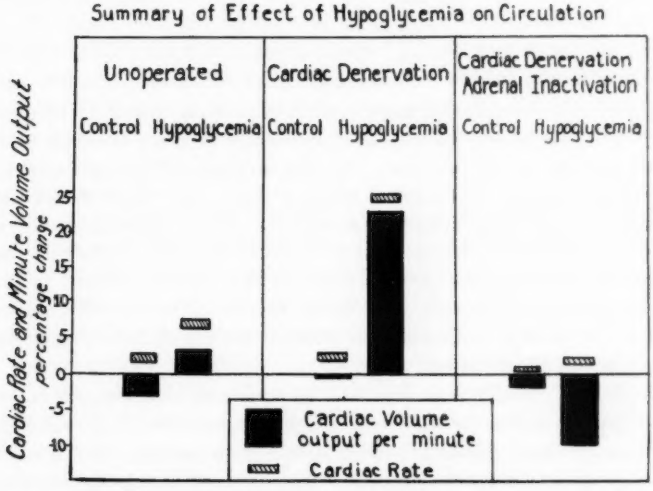


Fig. 3. Average percentage changes in cardiac minute volume output and ventricular rate in control experiments and during insulin hypoglycemia.

minute was recorded. The average change for this entire group was an increase of one beat per minute (1 per cent). Insulin hypoglycemia was attended by a rise in heart rate in four of seven normal animals and the average change for this group was an increase of ten beats per minute (7 per cent). The maximum change in heart rate in the control experiments consisted of an increase of fourteen beats per minute in one cat, and the average change in all control animals was an increase of four beats per minute (3 per cent).

The effect of insulin hypoglycemia on respiratory minute volume. The respiratory minute volume usually diminished during hypoglycemia in all three groups of animals. The average decrease was 67 cc. per minute

(11 per cent) in cats with denervated hearts and intact suprarenals, 60 cc. (12 per cent) in animals with denervated hearts and inactivated suprarenals, and 14 cc. (3 per cent) in normal cats. A small rise in respiratory minute volume was recorded in all the control animals but one, and the average increase for all control experiments was 56 cc. per minute (11 per cent).

The effect of insulin hypoglycemia on oxygen consumption. The amount of oxygen utilized per minute did not change to a significant extent during hypoglycemia in any of the three groups of animals. The largest average change recorded was a decrease of 1 cc. per minute (5 per cent) in the cats with denervated hearts and intact suprarenal glands.

The effect of insulin hypoglycemia on the oxygen content of arterial and mixed venous blood. The oxygen content of the arterial and mixed venous blood usually increased during hypoglycemia in the normal cats and in the animals with denervated hearts and intact suprarenal glands. In the normal cats, the average increase in arterial content was 2.31 volumes per 100 cc. (17 per cent) and the average rise in venous content was 2.40 volumes per 100 cc. (27 per cent). In the animals with denervated hearts, the average increase in arterial content was 1.54 volumes per 100 cc. (14 per cent) and the average rise in venous content was 2.25 volumes per 100 cc. (32 per cent). In contrast to these results, hypoglycemia was seldom attended by a significant increase in the oxygen content of arterial or venous blood in cats with denervated hearts and inactivated suprarenal glands. The control animals also showed no significant change in the oxygen content of the blood.

The effect of insulin hypoglycemia on arteriovenous oxygen difference. During hypoglycemia the difference in oxygen content of the arterial and mixed venous blood decreased in six of the nine animals with denervated hearts and intact suprarenal glands. The average change in all nine animals was a decrease of 0.72 volume per 100 cc. (17 per cent). In contrast to this, the difference in arteriovenous oxygen content usually increased during hypoglycemia in the cats with denervated hearts and inactivated suprarenal glands, although frequently the change was not beyond the limits of error of the method. The average increase in this group of animals was 0.38 volume per 100 cc. (10 per cent). In the control observations and in the normal cats during hypoglycemia, the arteriovenous oxygen difference usually remained practically unchanged.

The effect of insulin hypoglycemia on the minute volume output of the heart. The volume output of the heart increased 46 to 415 cc. per minute during hypoglycemia in six of nine animals with denervated hearts but intact suprarenal glands, while a decrease was recorded in two of the remaining cats. The average change in the entire group was an increase of 23 per cent. In contrast to these results, the cardiac output diminished during hypogly-

cemia in seven of the nine cats with denervated hearts and inactivated suprarenal glands, while in the remaining two, a rise of 5 cc. and 68 cc. per minute was recorded. The average change in this group was a decrease of 10 per cent. An increase of 7 to 28 cc. per minute was observed during hypoglycemia in five of seven normal cats, and the average change for all seven animals in this group was an increase of 3 per cent. An average decrease of 2 per cent was recorded in the control animals. In only one of twelve control experiments did the cardiac output increase as much as 30 cc. per minute.

Discussion. The cardiac output failed to increase appreciably during hypoglycemia in all but one of the animals with denervated hearts and inactivated suprarenal glands. On the other hand, an increase was recorded in six of nine cats with denervated hearts but intact suprarenals.[1] These results indicate that the rise in minute volume output in the latter animals was due to sympathicosuprarenal stimulation. The mechanism of the increase in cardiac output, therefore, is the same as that which Cannon, McIver and Bliss (1924) demonstrated to be responsible for the rise in heart rate during hypoglycemia in similar animals.

An average decrease of 10 per cent in cardiac minute volume output was recorded during hypoglycemia in the animals with inactivated suprarenal glands, while in the control experiments on all groups of animals without hypoglycemia the average diminution amounted to only 2 per cent. These observations indicate that the decrease in the minute volume output recorded in the cats with inactive suprarenals resulted from a deleterious effect of hypoglycemia on the heart. In animals with denervated hearts and intact suprarenals, the effect of sympathicosuprarenal stimulation usually predominated over the depressive effect of hypoglycemia on the myocardium, and a rise rather than a decrease in cardiac output resulted. No explanation is available for the rather large increase in circulatory minute volume observed in one cat with inactive suprarenals. At necropsy the left suprarenal gland appeared to be completely denervated and no accessory glands were found. We do not feel, however, that this single exception weighs heavily against the general results of the investigation.

Dworkin (1931) demonstrated that hypoglycemia causes central vagus stimulation as well as sympathicosuprarenal excitation and that, in intact, unanesthetized cats, the antagonism of these two effects usually maintains the heart at a relatively constant rate. In cats anesthetized with amytal, however, hypoglycemia was attended by an increased heart rate, probably because of depression of the inhibitory effect of the vagus by the anesthesia.

[1] In two of the three animals with denervated hearts and intact suprarenal glands in which the cardiac minute volume output did not increase during hypoglycemia, the experiments were made three and five days respectively after cardiac denervation, and the animals had not recovered from the operative trauma.

In our experiments on animals that had not been operated upon, hypoglycemia caused a rise in heart rate of fourteen or more beats per minute in four of seven cats, and in three animals the cardiac output increased more than 20 cc. per minute. The failure of the heart rate and minute volume output to increase during hypoglycemia in certain of these animals may have been due to incomplete abolition of vagus effects by the anesthesia. In view of these observations, the rise in ventricular rate and cardiac minute volume output observed by Ernstene and Altschule (1931) in normal persons during hypoglycemia indicates that, in man, hypoglycemia causes predominant sympathicosuprarenal stimulation.

In this series of experiments on cats, the circulatory minute volume generally increased during hypoglycemia only in those animals in which the heart rate also increased. No constant relationship was noted, however, between the degree of increase in ventricular rate and the magnitude of the rise in cardiac minute volume output, nor was either change proportionate to the extent to which the blood sugar was reduced.

SUMMARY AND CONCLUSIONS

1. Measurements of the heart rate, respiratory minute volume, oxygen consumption per minute, and cardiac minute volume output were made before and during insulin hypoglycemia in three groups of cats: 1, normal animals; 2, animals with denervated hearts, and 3, animals with denervated hearts and inactivated suprarenal glands. Control experiments were made on cats in all three groups.

2. In the cats with denervated hearts and intact suprarenal glands, insulin hypoglycemia was attended by an average increase of 23 per cent in the minute volume output of the heart and an average rise in heart rate of thirty-four beats per minute (26 per cent). In contrast to this, in the animals with denervated hearts and inactivated suprarenal glands, hypoglycemia caused an average decrease of 10 per cent in cardiac minute volume output and an average increase in heart rate of but one beat per minute (1 per cent).

3. The results indicate that both the increase in cardiac minute volume output and the rise in ventricular rate during hypoglycemia in animals with denervated hearts and intact suprarenal glands result from increased discharge of adrenin in response to stimulation of the sympathetic nerves to the suprarenal glands.

4. The decrease in circulatory minute volume observed during hypoglycemia in cats with denervated hearts and inactivated suprarenal glands indicates that hypoglycemia *per se* has a deleterious effect on the myocardium.

REFERENCES

BOOTHBY, W. M. AND I. SANDIFORD. Basal metabolic rate determinations. W. B. Saunders Company, 1920.
CANNON, W. B., J. T. LEWIS AND S. W. BRITTON. This Journal **77**: 326, 1926.
CANNON, W. B., M. A. McIVER AND S. W. BLISS. This Journal **69**: 46, 1924.
DWORKIN, S. This Journal **96**: 311, 1931.
ERNSTENE, A. C. AND M. D. ALTSCHULE. J. Clin. Invest. **10**: 521, 1931.
FICK, A. Sitzungsberichte der phys.-med. Gesellsch. zu Wurzburg, 1870.
FOLIN, O. J. Biol. Chem. **77**: 421, 1928.
J. Biol. Chem. **81**: 231, 1929.
VAN SLYKE, D. D. AND J. M. NEILL. J. Biol. Chem. **61**: 523, 1924.

HOURLY VARIATIONS IN WEIGHT LOSS FOLLOWING INGESTION OF FOOD

CARL IVER HOVLAND

Institute of Human Relations, Yale University[1]

Received for publication November 21, 1934

The researches of Benedict and Joslin (2) and of Benedict and Root (3) have clearly demonstrated that the taking of food is an important factor influencing rate of weight loss.[2] In neither study, however, was the amount of food controlled nor stated. The present investigation aims to determine the effects of different quantities of food upon weight loss measured at hourly intervals after ingestion. Comparison of these records with those obtained in the study of variations in oxygen consumption following the taking of food should afford additional evidence concerning the relationship between these two measures of energy expenditure.

METHOD. Determinations of weight loss were made by means of a large balance, sensitive to 0.1 gram, of the type described by Benedict and Root (3). A large cot, upon which the subject reclined during the experiment, was mounted upon one beam of the apparatus, while counterbalancing weights were placed upon the other. Due to the difficulty of obtaining records exactly upon the hour, since the subject is losing weight continuously, the exact moment when equilibrium was reached was measured by means of a stop watch, and interpolations were made in the data.

Ten normal, healthy male subjects were employed. The age range was from eighteen to thirty-four years, with a mean age of 23.8 years. Their weights ranged from 132 to 178 pounds (mean 148.9 lbs.) and their height from 54 to 61 inches (mean 56.5 in.). The subjects ate a very light breakfast and then reported at the laboratory. They rested and relaxed until noon when the experiment began. Preliminary investigation showed that by noon our subjects had re-attained the rate of weight loss they had when measured in a basal state in the morning before breakfast.

[1] This experiment was carried out in the Psychology Laboratory of Northwestern University. The balance was loaned to Dr. G. L. Freeman through the kindness of Dr. F. G. Benedict. The assistance of Dr. G. L. Freeman is gratefully acknowledged.

[2] The term "weight loss" is used throughout this article in preference to the more common term "insensible perspiration." The latter term is inaccurate, since, as Dr. W. M. Boothby has pointed out in a communication to the writer, the weight loss is not entirely perspiration, but also carbon dioxide, and is not insensible, because it can easily be measured.

The experimental meal was given at twelve o'clock noon, immediately after a pre-ingestive rate (called "basal" in the tables and figure) had been established. Weight loss was then determined at hourly intervals thereafter until five o'clock. The subjects continued to recline upon their cot, but were permitted to read if they wished. No physical exertion was allowed. Light, loose clothing was worn. The shoes were removed. Prior instructions to the subjects prevented artificial influences, such as evaporation from moistened hair, expectoration, and the like, from influ-

TABLE 1

Weight loss (in grams) per hour for successive hours following the ingestion of a heavy lunch of three sandwiches and three glasses of milk

Averages based upon two day's records for ten subjects

	PRE-INGESTION (BASAL)	HOURS AFTER FOOD INGESTION				
		1	2	3	4	5
Grams lost per hour	34.47	38.49	40.01	42.36	38.46	36.10
Range (in grams)	26–44	32–46	34–45	35–48	32–46	31–44
Amount of change (in grams)		4.02	1.52	2.35	3.90	2.36
P.E.$_M$ of change		0.56	0.46	0.39	0.42	0.47
Percentages of base rate	100.00	111.66	116.07	122.89	111.57	104.73

TABLE 2

Weight loss (in grams) per hour for successive hours following the ingestion of a light lunch of one ham sandwich and one glass of milk

Averages based upon two day's records of ten subjects

	PRE-INGESTION (BASAL)	HOURS AFTER FOOD INGESTION				
		1	2	3	4	5
Grams lost per hour	33.84	36.51	37.13	35.40	34.39	34.21
Range (in grams)	27–46	29–44	31–44	30–41	31–40	29–38
Amount of change (in grams)		2.67	0.62	1.73	1.01	0.18
P.E.$_M$ of change		0.45	0.43	0.37	0.41	0.33
Percentages of base rate	100.00	107.89	109.72	104.61	101.62	101.09

encing the results. A large jar, mounted directly upon the cot, was used for the collection of urine. Room temperature was maintained between 68 and 70 degrees Fahrenheit.

Five days were used for the experimentation upon each subject. Monday was devoted to accustoming the subject to the routine and apparatus. One type of experimental meal was given on Tuesday and Thursday, and the other on Wednesday and Friday. Half of the subjects were given the light meal first, and the other half the heavy. Each reading in the follow-

ing tables is based upon twenty determinations (two days for each of ten subjects). The light meal consisted of a ham sandwich weighing 350 grams and a glass of milk weighing 220 grams. The heavy meal was made up by tripling the above rations.

EXPERIMENTAL RESULTS. The changes in weight loss from hour to hour following the heavy meal are presented in table 1. The range for the ten subjects and the probable error of the average changes give some idea of the individual differences. The percentages of the pre-ingestive (basal) rate are also given for each hour.

The data on weight loss changes following a light meal are presented in table 2. The averages are based upon the same ten subjects, two days, records being used for each.

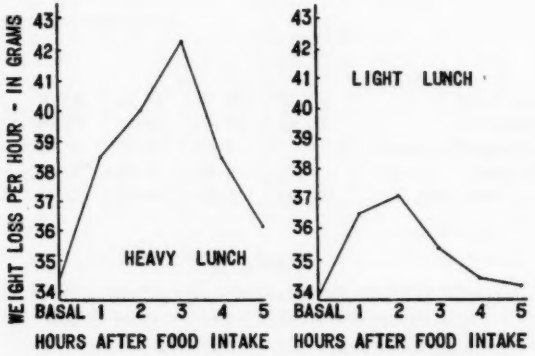


Fig. 1. Average number of grams of weight lost per hour by ten subjects following the ingestion of heavy meal, consisting of three ham sandwiches and three glasses of milk, and light meal, made up of one ham sandwich and one glass of milk. Pre-ingestive rate labelled "basal."

The results on the two different types of meal are pictured in the form of curves in figure 1.

DISCUSSION. It will be observed from table 2 and figure 1 that the normal course of weight loss following the ingestion of a small meal involves an increase in rate of loss for about two hours after food ingestion. After a heavy meal (table 1) the maximum rate is not reached until the third hour. Five hours after the light meal the weight loss is approximately equal to the pre-ingestive (basal) rate, but after the heavy meal a considerably longer period is required to return to the base rate, since by the fifth hour the rate is still considerably above that at the beginning. The maximum increase in the case of the light lunch is about ten per cent, while after the heavy meal the increase is as high as twenty-three per cent. These findings

parallel those of Grollman (4) to a striking extent. He found the maximum oxygen consumption at two hours after the light meal, and three hours after the heavy. His percentages of increase, however, are larger in the case of the heavy meal, due partly, presumably, to the larger meal taken by his subjects. Our percentages of increase are, however, of the order of magnitude reported by Benedict and Root (3). That the increased rate of loss is principally the effect of food and is not the result of other causes is established by unpublished data of the author for an experiment in which no food was given, and by the results of Benedict and Carpenter (1) in their studies on the effects of food upon metabolism with control periods of fasting. The close correspondence of our results to those obtained on oxygen consumption gives added evidence of the close relationship existing between weight loss and metabolism as measured by the traditional oxygen consumption method.

SUMMARY

The hourly loss of weight was recorded on two successive days for ten subjects following the ingestion of (*a*) a light lunch, made up of a glass of milk weighing 220 grams and a ham sandwich weighing 350 grams, and (*b*) a heavy lunch, consisting of triple rations of the above meal. After the light lunch there was a progressive increase in rate of weight loss for two hours, and then a decline which after five hours brought the weight loss back to the base rate. Following the heavy meal there was an increased rate of weight loss for three hours. Five hours after food the rate of loss was still nearly 5 per cent above the pre-ingestive rate. The maximum increase for the light meal was 10 per cent, for the heavy meal 23 per cent. These results are in close agreement with those of Grollman (4) on oxygen consumption following food intake. This similarity emphasizes the intimate relationship between these two indices of energy expenditure.

REFERENCES

(1) Benedict, F. G. and T. M. Carpenter. Carnegie Inst. Wash. Pub. 261, 1918.
(2) Benedict, F. G. and E. P. Joslin. Carnegie Inst. Wash. Pub. 176, 1912.
(3) Benedict, F. G. and H. F. Root. Arch. Int. Med. **38:** 1, 1926.
(4) Grollman, A. This Journal **89:** 366, 1929.

THE SUBNORMAL PERIOD OF NERVE RESPONSE

HELEN TREDWAY GRAHAM

From the Department of Pharmacology, Washington University School of Medicine, Saint Louis, Missouri

Received for publication November 19, 1934

The effect of yohimbine on isolated nerves seems to have been first investigated by Tait and Gunn (1908). They reported that when the nerve of a sciatic-gastronemius preparation from a green frog was exposed to a solution of a yohimbine salt, the preparation responded to indirect tetanic stimulation with a "fatigue tetanus," the height of which varied with the strength of stimulation and with the length of the preceding interval of rest (within limits, of course). Five seconds of rest was insufficient in some cases for complete recovery from such tetanic stimulation, while 0.25 second was the longest duration of the relatively refractory period following a single response. Tait the next year reported that this period may last 2.2 seconds, the absolutely refractory period being prolonged much less (maximal absolutely refractory period observed, 0.04 sec.). These remarkable results suggested that a study of yohimbinized nerve with the cathode ray oscillograph might yield information of value in the elucidation of the recovery process in nerve.

METHOD. The apparatus and methods used in this investigation have been described before (5). Inasmuch as the poisoned nerves showed long-lasting changes following a response, they were stimulated as few times as possible and at long intervals (1 sec. to 1 min., usually 4 sec.); this limitation of the number of observations decreased somewhat the accuracy of the determinations. Sciatic nerves removed from Rana pipiens or Rana catesbiana were employed, and were immersed in the desired solution for about half an hour before mounting, or were painted with solution after mounting in the moist chamber. The responses of the A group of fibres only were studied. The poisons used—yohimbine hydrochloride (1/100,000–1/5000), cocaine hydrochloride (1/50,000–1/5000), procaine hydrochloride (1/7,500–1/5000), quinine hydrochloride (1/5000–1/1000), strychnine sulfate (1/50,000–1/10,000), γ-4-morpholine propyl-α-naphthyl urethan hydrochloride (1/5000) (2)—were made up in Ringer's solution to the desired concentration, and were adjusted to neutrality. The experiments were performed at room temperature, which varied from 22° to 36° in the course of the whole series of experiments, but usually remained constant within one degree during an experiment.

RESULTS. Observations of the recovery of excitability by frog nerve at once confirmed Tait and Gunn's reports in general, but showed that there may be depression of excitability much later than 2 seconds after a single shock, and that this depression is not always continuous with the refractory period. With sufficiently dilute solutions (1/100,000 yohimbine hydrochloride) the course of recovery through the refractory and supernormal periods may in fact show little or no effect of the poison; and yet following this apparently normal recovery, the excitability will be slightly depressed for a number of seconds. This late subnormality, as it will be called in analogy with the well-known supernormality, may be both increased in degree and in duration by stronger solutions of yohimbine; in addition, however, such treatment lowers and shortens the supernormality, and also prolongs the refractory period throughout.

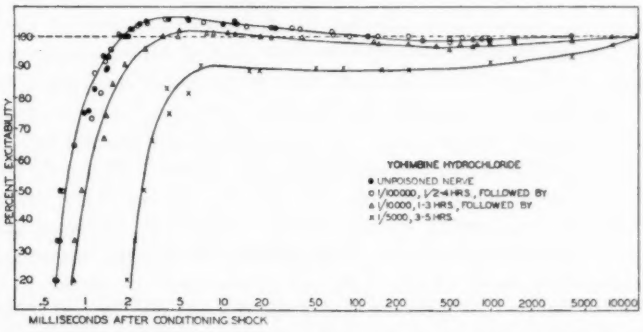


Fig. 1. Development of subnormality with yohimbinization. Green frog sciatic. Maximum spike height not decreased with 1/100,000; down to 73 per cent with 1/10,000; to 17 per cent with 1/5000. Temp. 27.8–29°C. 8/24/33.

The experiment plotted in figure 1 shows typical results with three yohimbine concentrations, applied in succession to the same nerve. As is shown in the figure, both the absolutely and relatively refractory periods are typically lengthened in all but the mildest degree of poisoning. Supernormality usually persists for a couple of hours with 1/10,000 to 1/20,000 yohimbine hydrochloride, but eventually may disappear with these concentrations, and it always goes fairly promptly after the application of 1/5000 yohimbine hydrochloride. When the supernormality is entirely gone, there remains in the recovery curve a crest of excitability corresponding to the former supernormal crest. This crest tends to fall lower and lower as well as later and later as the poisoning progresses, and eventually it becomes almost imperceptible in a plateau of excitability often extending beyond the region of former supernormality. The crest or plateau has only rarely been found to lie below 90 per cent of the level of resting excit-

ability. The period of rising excitability (5) tends to lengthen, as the delay in its start due to the prolongation of absolute refractoriness is more than compensated for by the delay of the attainment of the crest or plateau. Both before and after the crest falls below the resting level, it is frequently more prominent than in figure 1 (see for instance the excitability curves in figs. 4B and 5, the latter an unusually striking example).

With 1/10,000 to 1/20,000 yohimbine hydrochloride, the maximum degree of depression of excitability during the subnormal period is usually less than 5 per cent for the first couple of hours; with more intense poisoning it may increase to as much as 30 per cent (figs. 1, 2B-D, 4B, 5). The degree and the duration of the subnormality vary considerably from nerve to nerve and from time to time in the same nerve, even though the experimental conditions are not purposely varied. The asymptotic nature of the recovery from subnormality adds to the difficulty of definite determination of its duration. The duration of 12 seconds recorded in figure 1 is shorter than is frequently found in well-developed yohimbinization; in the experiment of figure 2B, 32 seconds were required for recovery of 100 per cent excitability. In general the duration probably tends to be greater with more intense poisoning, if the poisoning is not pushed too far; the duration is also increased by stimulation (see below). Not only the total duration of the subnormal period, but also the course of the excitability curve during this period varies widely. Maximum subnormality (or minimum excitability) occurs at progressively shorter intervals after the conditioning shock as the degree of poisoning is increased; in the experiment of figure 1, it advances from the 0.5 second interval it occupies with 1/10,000 yohimbine, to a 0.02 to 0.15 second interval with double this concentration. The logarithmic time scale of figure 1 tends to obscure the relative rate of change of excitability along different portions of the recovery curve; for this reason, particularly in order to show the form of the subnormal period more clearly, one of the curves of figure 1 has been reproduced in figure 2A on a linear time scale, and excitability curves from three other experiments with yohimbine hydrochloride have been plotted in figure 2B, C and D. On this linear scale the very early changes of excitability are too rapid to be represented, but the fall of excitability from its crest, wherever this may be, to its trough in the subnormal period is brought out clearly. The rate of fall seems to be determined to a considerable extent by the temperature, being much slower at 25.5° (B) than at 28° (A); an additional rise of 3° to 31° accelerates the rate somewhat further (C, D). From its minimum the excitability usually rises at first at about the rate at which it fell, but after it has risen perhaps half-way to its resting level, it slows up rather abruptly, and then completes its rise very slowly (fig. 2, A–C). Occasionally however, the excitability remains for

some time at or near its minimum, from which it rises only very gradually (fig. 2D). Temperature does not seem to play a deciding rôle in determining the character of the rise; figure 2, C and D represent experiments performed at the same temperature (31°C.).

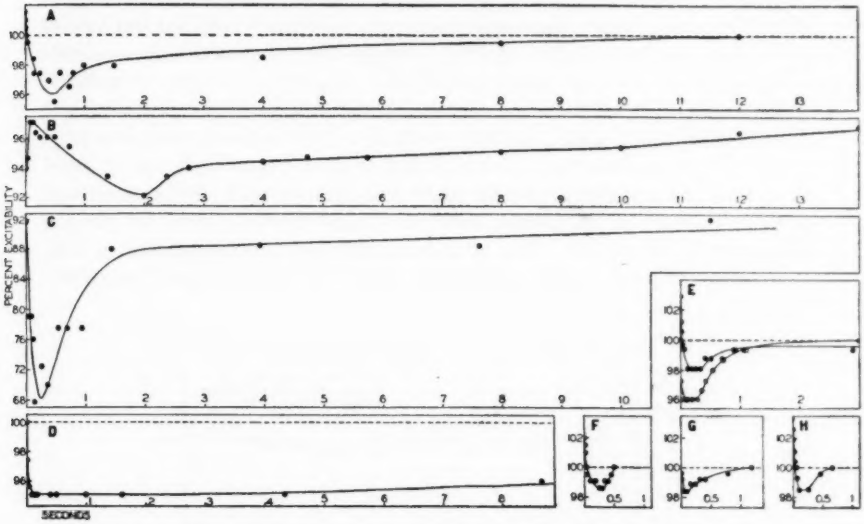


Fig. 2. Form of subnormal period. In all cases except C, poisons painted on mounted green frog sciatic nerves; in C, bull frog sciatic soaked before mounting.

A. 1 to 3 hours after yohimbine hydrochloride (1/10,000) following 1/100,000 for 4 hours. Temp. 28°C. 8/24/33.

B. 2 to 4 hours after yohimbine hydrochloride (1/10,000). Temp. 25.5°C. 10/6/33.

C. Soaked 1 hour in yohimbine hydrochloride (1/20,000), data recorded within following 3 hours. Temp. 31°C. 7/14/34.

D. 1 to 3½ hours after yohimbine hydrochloride (1/15,000). Temp. 31°C. 7/12/34.

E. Two degrees of poisoning with strychnine sulfate. ●, 1–2 hours after 1/20,000 subsequent to 1/50,000 2 hours previously; ○, 4–5 hours after 1/10,000, following above. Temp. 27–28°C. 10/4/34.

F. 1 to 3 hours after cocaine hydrochloride (1/20,000). Temp. 28°C. 9/26/33.

G. 1 to 3 hours after procaine hydrochloride (1/5,000). Temp. 25°C. 9/28/33.

H. ½ to 3 hours after quinine hydrochloride (1/5,000). Temp. 24°C. 10/2/33.

In some of these experiments on yohimbinized nerves, the conditioning and testing shocks were applied at the same pair of electrodes; in others the shocks were applied at different electrodes, to avoid complications from local effects; and in still others the subnormality was recorded with both

arrangements. The separation of the two shocks did not seem to make any significant difference in the degree or duration of the late subnormality of excitability (see below for local effects on recovery of height).

Yohimbine is the only substance yet tried which makes possible a period of subnormality lasting so many seconds after a nerve response, but there are a good many other substances which produce a similar but briefer condition. Apparently the ability to change the nerve so that it manifests subnormality is a fairly widely-distributed property; it seems for instance to be possessed by all local anesthetics, though not by the general aliphatic anesthetics (ether, amyl alcohol, urethan). Strychnine sulfate has been found a very satisfactory means of inducing the manifestation of brief (1–3 sec.) subnormality without great interference with the supernormal period; examples of the results with strychnine-treated nerves are plotted in figures 2E, 3 and 4A. Due to the maintenance of supernormality with this drug, except in intense poisoning, there is a wide swing of irritability following a response.

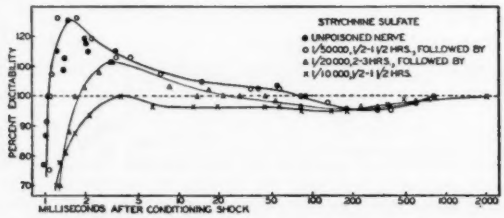


Fig. 3. Development of subnormality with strychninization. Green frog sciatic. Maximum spike height not changed with 1/50,000 nor at first with 1/20,000; later, down to 65 per cent with 1/20,000; 27.5 per cent at end of experiment. Temp. 33.5°–35°C. 7/26/34.

The brevity of the subnormal period manifested by strychnine-treated nerves is due primarily to a more rapid over-all rise from the minimum excitability, which falls but little earlier than with yohimbinized nerve at corresponding temperatures (cf. fig. 2, A and E). The resultant trough of subnormality manifested by strychninized nerves therefore apparently corresponds to the early sharp dip usually observed with yohimbinized nerves. The more prolonged portion of subnormality induced by yohimbinization seems to be absent or at least much less marked in subnormal periods induced by treatment with strychnine. Because of the rapid disappearance of the subnormality, the cycle of supernormality—subnormality can be repeated more often in strychninized than in yohimbinized nerve; and for the same reason, in order to demonstrate summation of subnormality (see below), it is necessary to stimulate a strychninized nerve more rapidly than a yohimbinized nerve.

Nerves treated with a quinine salt or a local anesthetic (the hydrochlorides of cocaine, procaine, and γ-4-morpholine propyl-α-napthylurethan have been tried) resemble strychninized nerves in the brevity of their subnormal periods (fig. 2, F–H). The subnormality seems to reach its minimum more promptly with these local anesthetics than with yohimbine at the same temperature, and in general supernormality is likely to be somewhat less than after strychnine or yohimbine treatment.

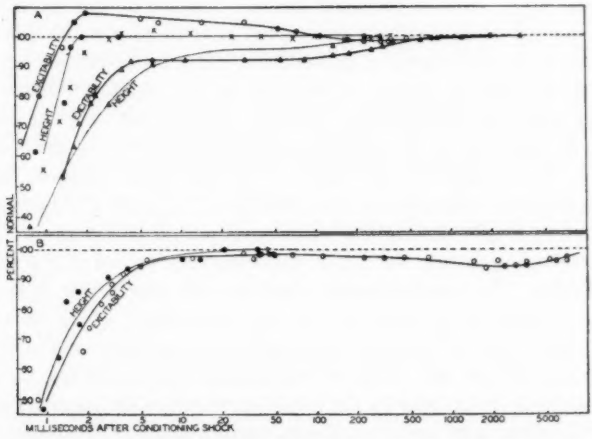


Fig. 4. Recovery of height and of excitability in nerves manifesting subnormality. Green frog sciatic nerves. Conduction distance 8.5 mm. for testing shock, 19–23 mm. for conditioning shock. Outline symbols and heavy curves, excitability; solid symbols and light curves, height.

A. ○, ●, 1–2 hours after 1/50,000 strychnine sulfate painted on; △, ▲, 1–3 hours after 1/10,000 applied subsequent to above; ×, excitability after 1½ hours soaking in Ringer's solution following this strychninization. Maximum spike height unchanged with 1/50,000, down to 54 per cent with 1/10,000; not raised by washing. Temp. 32°C. 7/27/34.

B. 1 to 3 hours after 1/10,000 yohimbine hydrochloride painted on (1/20,000 applied 4 hours before 1/10,000). Height determinations throughout subnormal period showed 100 per cent recovery. Maximum spike height at end, 92 per cent of that before poisoning. Temp. 22°C. 10/9/33.

Because of the obvious resemblance of subnormality to refractoriness, characteristics of the refractory period other than depression of excitability were looked for during the subnormal period. The most obvious of these characteristics, depression of height of response, was never found during the subnormal period when this period was preceded by a rise of excitability up to or above its resting level. (Decrease of height of response was tested for with supermaximal testing shocks, following maximal

conditioning shocks, in order to avoid all complications arising from decreased excitability.) In such cases, complete recovery of height of response always occurred simultaneously, within the limits of experimental error, with 100 per cent recovery of excitability (fig. 4A, upper curves; the slight difference in the interval required for 100 per cent recovery of height and of excitability here may be explained by the fact that the height measurements were made somewhat later than those of excitability). The relationship between recovery of excitability and recovery of height was thus exactly the same as in unpoisoned nerve. Failure of the spike height to decrease during the late subnormal period is analogous to its failure to increase during the supernormal period (5). The fact that the method for measuring changes of height of response is less sensitive than that for measuring changes of excitability, and the further fact that the changes of height to be expected in the subnormal period would not be great when the depression of excitability is as slight as in these cases, suggest caution in the interpretation of these negative findings. It is certain, however, that any change in height during the subnormal period is less than the height decrease occurring during the relatively refractory period at the same level of irritability. The experimental findings on which this statement is based are illustrated in figure 4. In the experiment of figure 4A, while supernormality was still present, no height measurement was made during the refractory period at a level of excitability corresponding to the subnormal minimum; but later in the experiment when the excitability at 0.3 second had fallen to 95 per cent, the maximum height of response was still found to be 100 per cent at this interval. It is obvious from the figure that earlier, when 95 per cent excitability was still attained during the refractory period, it would have corresponded to a decreased height well within the limits of observation. Further, figure 4B shows that if at the interval (2–3 sec.) marked by the subnormal minimum of excitability, a decrease of height had occurred comparable to that at the same excitability level during the refractory period, it could not have escaped observation.

Figure 4B and the lower curves of figure 4A illustrate the cases in which the excitability fails to reach 100 per cent before the subnormal period. In B the excitability rises very close to 100 per cent and the height recovers completely in the interval required for the excitability to reach its early crest. Since this interval (0.025 sec.) is rather long for the period of rising excitability, it appears that there has been some delay in the final stages of recovery from refractoriness. Such delay must be enormously increased in later stages of poisoning, when it is accompanied by marked prolongation of the early part of the refractory period; in these cases complete recovery of height falls much later than the early crest of excitability, but much earlier than the final attainment of 100 per cent irritability. At the end of

the experiment plotted in figure 1, complete recovery of height required 0.062 second, while in the more advanced degree of poisoning plotted in figure 4A, complete recovery of height required 0.30 second.

Occasionally, in deeply poisoned nerves which had been subjected to high room temperatures and considerable stimulation, a supermaximal testing shock applied through the electrodes used for the conditioning shock, produced a response during the subnormal period lower than the response produced at shorter intervals between the shocks. In every case, however, this seems to have been due to exhaustion of the nerve by the experimental procedures, and not to true late subnormality of height of response. It was never observed when the two shocks were applied at different points on the nerve.

Another characteristic of the relatively refractory period is decreased rate of conduction of the impulse. In eight experiments (2 with green frog nerves, 6 with bull frog nerves; conduction distances 95 to 210 mm.),

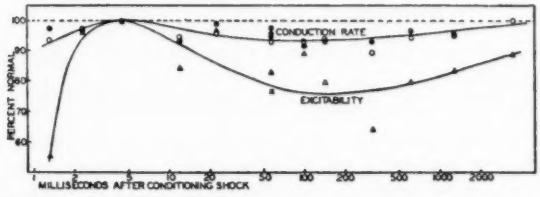


Fig. 5. Conduction rate in subnormal period. Bull frog sciatic, ½–5 hours after 1/10,000 yohimbine hydrochloride painted on, following 1/20,000 5 hours earlier. Conduction distance for both shocks 32 mm. or 210 mm. Conduction rate: ○, 210 mm. to lead; ●, 210 mm. to 32 mm. from lead. Unconditioned conduction rate, 51.5–49.2 m./sec. Temp. 32°C. 7/9/34.

the conduction rate was measured at various intervals during the recovery processes following a response. The rate showed the familiar decrease during the refractory period, an increase during the supernormal period, and a second decrease during the subnormal period. The results of one of the experiments on bull frog nerve are plotted in figure 5. In this experiment the conditioning and testing shocks were applied at the same point on the nerve, either 32 or 210 mm. from the ground lead of the amplifier, and the time for conduction over these two distances observed with various intervals between the shocks. The changes in rate of conduction from the farther stimulating point to the leading off electrode, and from the farther to the nearer stimulating point, have been plotted; the changes are so nearly alike in the two cases that only one curve has been drawn through the two series of points. The irritability and the conduction rate in this experiment rose just to normal prior to the subnormal period; during the subnormal period, the slowing of conduction in general ran paral-

lel to, but was less than, the degree of subnormality in all these experiments. The testing and conditioning shocks were usually applied at the same electrodes, but the slowing of conduction was also observed when they were applied at separate pairs of electrodes. The increased conduction time is clearly not due to local changes of any kind, but to actual slowing of the impulse. Similar slowing has been observed by Gasser during the subnormal period following tetanic stimulation of unpoisoned nerve.

Repeated stimulation of yohimbinized nerves at appropriate intervals (0.4–6 sec. intervals) results in increased depression of excitability during the subnormal period; strychninized nerves show the same effect with the shorter intervals only. At first each shock of an appropriately timed

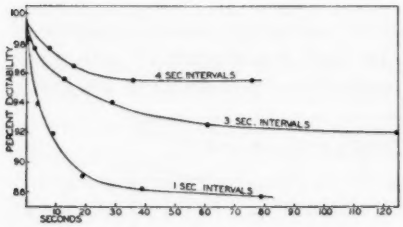


Fig. 6

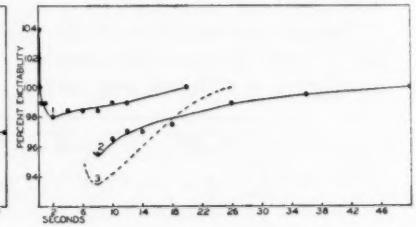


Fig. 7

Fig. 6. Effect of repeated stimulation on degree of subnormality. Green frog sciatic, ½–3 hours after yohimbine hydrochloride painted on (1/10,000). Excitability determined 0.082 second after last conditioning shock of series of indicated length and frequency. Temp. 29°C. 9/19/33.

Fig. 7. Comparison of subnormality after a single conditioning shock and after several conditioning shocks. Green frog sciatic, 1–5 hours after yohimbine hydrochloride (1/20,000) painted on. Curve 1 = subnormality observed after 1 conditioning shock; curve 2 = subnormality observed after 4 conditioning shocks at 2 second intervals; curve 3 = geometrical construction (derived from curve 1) of subnormality following 4 conditioning shocks at 2 second intervals. Temp. 23°C. 10/7/33.

series adds to the depression produced by the preceding shocks, but recovery from the depression eventually just balances the increments of depression, so that the excitability reaches a level below which it does not fall during continued stimulation at a constant rate. This level is lower with faster rates of stimulation; figure 6 presents the results of an experiment in which a yohimbinized nerve, showing a depression of excitability to 99.4 per cent of its resting value 0.082 second after a single conditioning shock, was subjected to a series of such shocks at 1, 3 or 4 second intervals. 0.082 second after the last of varying numbers of shocks at each interval, the excitability was tested, and found to decrease as indicated by the points on the curves; the levels eventually reached and maintained were 87.5, 92, and 95.5 per cent respectively with the three intervals.

It is obvious that if the subnormality following each conditioning shock of a series were exactly like that following a single shock, and if each one ran its course independent of its predecessors and successors, the depression of excitability produced would be in general similar to that observed. Geometrical summations of observed individual subnormalities however always produce greater depression of excitability than is observed in series stimulation, and the course of recovery observed when the series is interrupted is always slower than would be predicted from such summations. These points are illustrated in figure 7. The latter part of the recovery following a single shock was reproduced in line 1, while line 3 was derived by plotting four such recoveries at 2 second intervals. At the start line 3 falls well below line 2, the recovery actually observed after 4 conditioning shocks, but line 3 ends much earlier. Such results combine with other similar evidence to indicate that with repeated stimulation, the subnormality following a single shock tends to grow less in degree and longer in duration.

As would be expected, the degree of depression of irritability by series stimulation at a given rate tends to be greater in nerves showing greater subnormality following a single conditioning shock. In intensely poisoned nerves it is possible by repeated stimulation to drive the excitability down to levels much below those illustrated, actually to a few per cent of its resting value. In these cases, however, the supernormal period has disappeared, and with it all possibility of a clear-cut temporal distinction between the refractory and subnormal periods. In the curve marked 1/5000 in figure 1, for instance, it is obvious that these two periods overlap, and when the poisoning has progressed still further than in this case, the possibility that after stimulation refractoriness extends even several seconds beyond the last conditioning shock of a series, can not be ruled out.

The above description of the effects of yohimbine, etc., on nerve is confined to effects on the recovery function of nerve, and it is in these functions that the chief interest lies. The other effects of the drugs may however be briefly summarized. With yohimbine treatment, the height of the spike tends to fall, reaching about 90 per cent of its unpoisoned level with 2 to 3 hours of experimentation after the application of 1/10,000 yohimbine hydrochloride. With more dilute yohimbine solutions, the spike height may be maintained for hours almost as well as in an unpoisoned nerve; 1/20,000 strychnine sulfate, 1/10,000 cocaine hydrochloride, and 1/5000 procaine hydrochloride or quinine hydrochloride were found no more toxic to the spike than 1/10,000 yohimbine hydrochloride; the minimum concentrations of all these substances which are effective in inducing the manifestation of subnormality depress the spike but little. The resting (unconditioned) excitability may be decreased 0 to 20 per cent in the course of a long experiment with 1/10,000 yohimbine hydrochloride or cocaine hydrochloride;

quinine is probably less, and procaine probably more, depressant of the excitability in the concentrations used for subnormality experiments. Low concentrations of strychnine sulfate tend to increase the unconditioned excitability while at the same time inducing the manifestation of subnormality; stronger solutions produce a fall of excitability. The rate of conduction may not change during an experiment with 1/10,000 yohimbine hydrochloride, or it may be decreased by as much as 20 per cent; the other drugs also all tend to decrease the conduction rate in the concentrations used.

The changes induced in the nerve by these drugs—decrease of spike height, of excitability, of conduction rate and of supernormality, increase of refractoriness and subnormality—are all more or less reversible when the nerve is washed with Ringer's solution, provided the intoxication has not been too severe. The effects of yohimbine and of quinine are more difficult to reverse than those of the other drugs; and the restoration of the spike height is perhaps more difficult than that of the other functions. The ease with which the strychnine effect on the recovery of nerve is reversed is illustrated in figure 4A. After the intoxication had been pushed to the marked extent indicated by the lower curves, the nerve was soaked in strychnine-free Ringer's solution. The figure shows that this treatment removed the subnormality almost completely, and restored the supernormality to some extent. The strychninization and experimentation had decreased the spike height to 54 per cent, the irritability to 63 per cent, and the conduction rate to 74 per cent of their respective values in the unpoisoned nerve; the treatment with Ringer's solution restored the irritability and the conduction rate to practically their original values, but the spike height in this case continued to fall in spite of the restorative treatment.

Discussion. Comment on the above experimental results will be confined to discussion of four questions.

1. Is late subnormality simply a prolongation of the relatively refractory period? This is the interpretation given the yohimbine effect by Tait and Gunn. The intervention of the supernormal period between the refractory and subnormal periods suggest that this interpretation is incorrect; yet, except in the occasional cases where the supernormal course of the excitability curve is not modified by the appearance of subnormality, it is quite possible to regard the two periods of depressed excitability as one long, continuous phenomenon, preceding, accompanying (and modifying), and following the supernormal period. When the results on recovery of height of response are taken into consideration, however, it becomes clear at once that subnormality cannot be prolonged refractoriness, for the final recovery of height always occurs long before that of excitability when subnormality is present. One of the characteristics of the refractory period, depressed height of response, is therefore always absent in the latter part of the subnormal period, and is absent during the whole period when

the period is separated from the refractory period by an intervening rise of excitability to or above its resting value. When the excitability fails to reach this level before the subnormal period, the time required for recovery of height may be little or much longer than the period of rising excitability in unpoisoned nerve. The simplest interpretation of the facts in these cases is that complete recovery of height marks the end of the more or less prolonged refractory period. This interpretation is strengthened by the fact that the absolutely refractory period and the immediately subsequent portion of the recovery curve are delayed when the recovery of height is delayed. It thus appears that, although subnormality is not prolonged refractoriness, refractoriness may be prolonged well into the subnormal period.

Further evidence against the identity of refractoriness and subnormality is found in the experiments with repeated stimulation. When a nerve is made to respond during its relatively refractory period, the second response wipes out, so to speak, the residual refractoriness of the first response; and the nerve recovers from the second response along exactly the same curve as if there had been no preceding response (6). In other words, refractoriness does not sum. The fact that subnormality from successive stimulations does add up is obvious from the experiments with poisoned nerve described above; moreover, the development of detectable subnormality in unpoisoned nerve with tetanic stimulation (see below) is very likely due to such summing of subnormality.

2. How early in the nerve response does the process manifested as subnormality start? In the phenomenon chiefly studied here—the response of A fibres in poisoned nerves to single shocks—the first sign of subnormality occurs in the period following supernormality; between the two there is sometimes an indifferent region where the excitability may be indistinguishable from normal, but it is by no means certain that this region represents anything other than changes in excitability below the limit of observation. However this may be in cases of very mild poisoning, it is evident that as the poisoning progresses, the process underlying subnormality must begin very early indeed. For instance in those cases where the recovery of height of response requires little more time than the normal period of rising excitability, it is evident that depression of excitability occurring at this interval as well as later, can not be regarded as due to refractoriness in the conventional meaning of the word, but must be due to subnormality. In view of the relation between positive after-potential and subnormality (3), an early start of subnormality is also suggested by Bishop's finding in C fibres of after-positivity without evidence of preceding after-negativity. It is in fact impossible to set a limit prior to which the process manifested as subnormality can not begin, and it may well start as early as the absolutely refractory period.

3. Does subnormality occur in unpoisoned nerve after a single stimula-

tion? In the course of the above experiments, repeated attempts to find it after single shocks have always failed prior to the poisoning, but the behavior of stimulated nerves suggests that it may nevertheless be present to an extent too small to be detected after a single spike. Recent work by Gasser has definitely proved that during the positive after-potential manifested by frog nerve following tetanic stimulation, there is a period of depressed excitability, and the increased positive after-potential induced by yohimbine (4) completes the proof of the relationship between after-positivity and subnormality. Either subnormality of very low degree occurs in normal nerves following a single shock, or else subnormality develops whenever the after-positivity is increased, whether by stimulation or by the application of a poison, above a certain value.

4. What is the bearing of these results on the theory of the recovery process in nerve? The existence of the subnormal period provides additional evidence of the duration and complexity of the recovery process occurring in nerve, probably after even a single spike. Regarding the particular recovery process associated with subnormality, the experimental results reported here suggest that like the other known recovery processes, it must begin before the spike potential has completely subsided; and that, like the process associated with supernormality, it does not affect the ability of the effectively-stimulated nerve to produce a spike.[1] It further appears that the process associated with subnormality may itself be a complex, with two more or less independent components differing from each other in rate of development. A similar suggestion of the complexity of the process arises from the shape of the positive after-potential observed after tetanic stimulation of unpoisoned nerve (3); the results with poisoned nerve make it clear that this complexity is not associated with the repetition of stimulation. That the process is not "all-or-nothing" is probably too obvious to require exposition.

SUMMARY

After treatment with yohimbine, strychnine, quinine, cocaine, or any one of various local anesthetics, the isolated sciatic nerve of the frog manifests after a single stimulation not only the refractory period and the supernormal period, but likewise a later period of subnormal excitability. The subnormal period first becomes visible at the end of the supernormal period, but gradually encroaches upon the latter and eventually becomes continuous with the prolonged refractory period. The maximum degree

[1] An increase of spike height during the positive after-potential following tetanic stimulation, has been reported by Woronzow; he attributes the increase chiefly to a decrease of the second phase of the response as a result of the stimulation, and no corresponding increase has ever been observed during the subnormality following a single shock applied to poisoned nerve.

of subnormality after a single shock varies from $\frac{1}{2}$ to 30 per cent of the resting level of irritability and occurs 0.1 to 3 seconds after the shock; the duration of the subnormal period varies from 1 to 30 seconds. Yohimbine induces more prolonged subnormality than do the other poisons.

During the subnormal period the maximum height of response is unchanged, but the conduction rate is decreased. The subnormality of conduction rate is less than that of excitability.

Repeated stimulation at intervals not exceeding the duration of the subnormal period increases the degree and duration of the subnormality.

The poisons inducing the manifestation of subnormality tend to depress the spike height, the conduction rate, and (except strychnine) the resting excitability.

Subnormality is not identical with refractoriness, but the process associated with it probably begins very early in nerve response. This process, which may actually be a complex of processes, is regarded as a normal constituent of the recovery of nerve.

REFERENCES

(1) Bishop. J. Cell. Comp. Physiology **5**: 151, 1934.
(2) Gardner, Clarke and Semb. J. Am. Chem. Soc **55**: 2999, 1933.
(3) Gasser. This Journal (in press).
(4) Graham and Gasser. Proc. Soc. Exp. Biol. Med. **32**: 553, 1934.
(5) Graham. This Journal **110**: 225, 1934.
(6) Kato, G. Further studies on decrementless conduction. Tokyo, 1926, p. 112.
(7) Tait. Quart. J. Exp. Physiol. **2**: 157, 1909.
(8) Tait and Gunn. Quart. J. Exp. Physiol. **1**: 191, 1908.
(9) Woronzow. Pflüger's Arch. **206**: 1, 1924.

THE INFLUENCE OF PARATHORMONE ON THE NEUROMUSCULAR SYSTEM: AN EXPERIMENTAL ANALYSIS[1]

ERNST GELLHORN

From the Department of Physiology of the University of Illinois, College of Medicine, Chicago

Received for publication November 1, 1934

In spite of the voluminous literature on the effects of parathormone and on the consequences of the removal of the parathyroid gland, our knowledge as to what happens in the muscle under these circumstances is very limited and not without contradictions (cf. Trendelenburg, 1934). For this reason a systematic study of the effect of parathormone on the neuromuscular system was undertaken.

The method employed in this paper is based on the observation that striated muscle, including its neuromuscular junction, is highly sensitive to various agents, particularly calcium, when it is in a hypodynamic state and the blood vessels of the muscle are perfused with the agents under investigation (Gellhorn, 1931–33; Taubmann, 1931). The general sensitivity of such preparations and the inherent quantitative possibilities of the method render it possible to investigate the action of parathyroid extract on a nerve muscle preparation.

Pithed frogs of medium size (Rana esculenta) were used, and the hind legs were perfused from the abdominal aorta. In a group of experiments two cannulae were introduced into the iliac arteries in order to perfuse each leg separately. Strongly buffered Ringer-phosphate solutions were used. The composition was 90 parts NaCl 6.0, KCl 0.075, $CaCl_2$ 0.075, distilled water ad 1000.0 and 10 parts of a mixture of $\frac{m}{10}$ primary and secondary sodium phosphate which had to be varied in order to give the proper hydrogen ion concentration for the several solutions. This control solution was exchanged for similar solutions containing various amounts of parathormone. The perfusion rate was kept constant throughout the whole experiment. The pH of the solutions was regularly adjusted to 7.2. The tendon of the gastrocnemius was connected to an isotonic lever recording the contractions on the kymograph. The magnification was sevenfold. The load was varied in experiments with different frogs according to the

[1] This paper was awarded the Alvarenga prize of the College of Physicians of Philadelphia for 1934.

Aided by grants of the National Research Council, and the Graduate School of the University of Illinois.

size of the frog and the fatigability of its muscles. The nerves were stimulated by rhythmic condensor discharges (maximal currents) with the apparatus of Schmitt and Schmitt (1932). First, a stimulation of 40 discharges per minute was used until the height of the contraction was reduced about 50 per cent. After an interval of 15 to 30 minutes the nerves were stimulated with a low frequency (15 per minute) and the experiment was started after the muscles had reached a rather steady state (relative fatigue) in which the height remains relatively constant over long periods of time. More than 500 experiments were performed.

RESULTS. PART I. *The effect of parathormone on the muscle in the hypodynamic state.* Preparations obtained from Eli Lilly & Company and Parke Davis & Company were used.[2] The former contains 20 Collip units per cubic centimeter, the latter 150 Hanson units. These solutions were diluted in the ratio of 1:100, 1:250, and 1:1000 with phosphate-buffered Ringer solution and exchanged for parathormone-free Ringer in the perfusion experiments.

In several hundred experiments it was found regularly that parathormone increases the height of contraction of the fatigued muscle. These effects are always reversible and increase with increasing concentration of the parathyroid extract. They may amount to 400 per cent and more. Parathormone 1:1000 displays a rather weak effect and this may be missing in some preparations, although in particularly sensitive preparations very strong effects may occasionally be observed. The effect starts after 2 to 5 minutes, the latent period depending mainly on the concentration of parathormone and the perfusion rate. The latter was always kept constant throughout the whole experiment. There was no indication of an influence of the various parathormone preparations on the diameter of the blood vessels. The effect was not due to pH, since this was always controlled. Neither was it due to phenol, because phenol-free extracts gave similar results and phenol in the concentration present in the extracts had no effect on the muscular response.

Whereas the experiments reproduced in plate I (figs. III and IV) show that parathormone increases the height of contraction of an indirectly stimulated perfused muscle which is rhythmically stimulated without any interruption, another group of experiments shows the influence of parathormone on the recovery of a previously fatigued muscle. In these experiments the nerve-muscle preparation was brought into a state of fatigue as described above. It was then subjected to alternate 10 minute periods of stimulation and 30 minute periods of rest, the perfusion fluid being changed by regularly alternating Ringer and Ringer plus parathor-

[2] The author wishes to express his thanks to both companies and particularly to Mr. H. W. Rhodehamel of the Eli Lilly Company for his friendly coöperation.

mone. A typical experiment (plate I, fig. I) shows how much the recovery is improved by allowing the preparation to be perfused by parathormone-Ringer. In spite of the progressive changes in muscular fatigue, which are apparent by comparison of subsequent Ringer periods or subsequent parathormone periods, a parathormone period shows regularly a greater height of contraction and, therefore, a larger amount of work than was obtained in the preceding Ringer period.

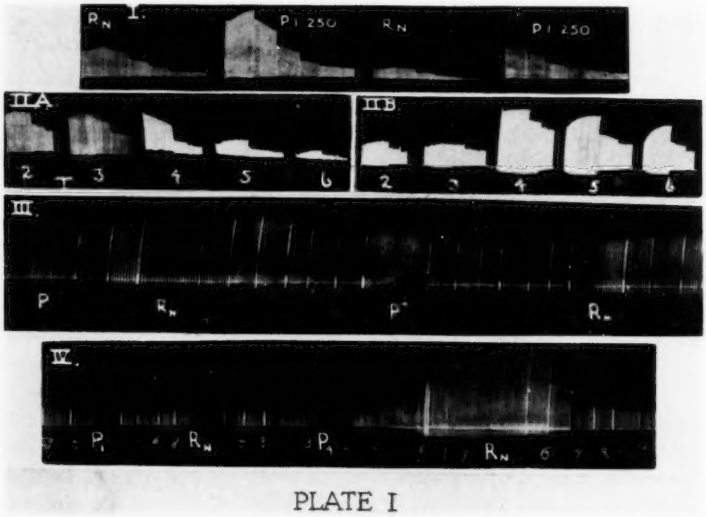


PLATE I

Plate I. Indirect stimulation of the muscle. Fig. I. Frequency of stimulation of the nerve: 45 per min. Interval between the individual stimulation periods 30 minutes. R_N = Ringer. P = Ringer + Parathormone Lilly 1:250.

Fig. II. Double perfusion preparation. IIa = perfusion with Ringer. IIb = perfusion with Ringer during the first two periods; (no. 2 and 3) hereafter with Ringer + parathormone 1:250. Stimulation period 5 minutes, and rest periods 5 minutes, alternate.

Fig. III. P = parathormone 1:250. R_N = Ringer. P'' = parathormone 1:250 which had been boiled in 10 per cent HCl for one hour.

Fig. IV. P_1 = with ethyl alcohol inactivated parathormone 1:200. P_4 = parathormone 1:200 (control). R_N = Ringer.

A similar result was obtained by a third method in which two cannulae were introduced in the iliac arteries, two legs being perfused separately. The muscle contractions caused by the rhythmic stimulation of the sciatic nerves of either side were simultaneously recorded on two kymographs. First, several periods were recorded during which the muscles were perfused with Ringer; thereafter, one muscle was perfused with Ringer-parathor-

mone, whereas in the other leg the perfusion with Ringer was continued. Figure II, plate I, shows a typical result of such an experiment. It is obvious that in contrast to the progressive and large decrease in height of contraction in the Ringer-perfused muscles (fig. IIa) their mates treated with parathormone showed remarkable recovery (fig. IIb). Such results distinctly confirm the above effects of parathormone on the height of contraction and the recovery from fatigue, and also indicate that the course of fatigue, which may be judged from the decline in the height of contractions in subsequent Ringer periods, is greatly delayed in the presence of parathormone.

PART II. *Is the effect of parathyroid extracts due to the specific hormone or to nonspecific impurities?* Since the parathormone now available is impure, any reaction which it produces may be due to parathormone or to non-specific substances. To investigate this possibility the extracts were subjected to various procedures which, according to observations of other investigators, influence the activity of the extract. In another group of experiments the results obtained with our method were directly compared with the blood calcium-raising action, which is ordinarily used in the standardization of parathormone.

According to Thomson and Collip (1932), boiling of the extract for one hour does not inactivate parathormone. Our experiments show that under these circumstances the increase in the height of contraction occurs as with the untreated parathormone. However, they state that boiling for one hour in 10 per cent HCl inactivates parathormone. An extract so treated and restored to neutrality, caused no increase in the height of contraction, although the nerve-muscle preparation reacted before and afterwards in the typical fashion (plate I, fig. III).

It was found by Tweedy that parathormone may be inactivated by ethyl alcohol. This method of inactivation was examined in the following manner. To a sample of parathormone (Lilly) 1.7 volume of 95 per cent ethyl alcohol was added. HCl was added to the mixture so that its concentration was 0.25 per cent. This stood at room temperature over night. The alcohol was then removed in vacuo and the sample was diluted with water to the original volume. Then the pH was adjusted and the preparation appropriately diluted with phosphate-Ringer. The result of the test was that, depending on the degree of dilution and the sensitivity of the preparation, the inactivated parathormone showed either no effect at all or a very slight effect, whereas the control experiment gave the typical response. Figure IV (plate I) shows quite plainly the enormous difference between inactivated and the active extract.

Furthermore, two extracts were compared in regard to their effect upon the nerve-muscle preparation, which extracts, according to the blood calcium test, contained the same number of units per cubic centimeter but

differed in the concentration of solids by about 100 per cent. Extract A contained 1.1 per cent solids and extract B 2 per cent, but both had 17 units parathormone per cubic centimeter. The effect of both extracts upon the muscle was exactly the same, which seems to indicate again that the hormone as it is tested by the blood calcium method is quantitatively related to the neuromuscular action of parathyroid extracts, and that the concentration of nonspecific substances is immaterial.

It is known (Taubmann, 1931) that calcium produces a similar change in the height of contraction of the perfused nerve-muscle preparation as parathormone does according to our own investigations. Therefore, the question arises whether the parathyroid extract contains some Ca which might account for the observed effect. Several facts exclude this possibility. First, the above mentioned procedures of inactivating parathormone do not alter the calcium content of the extract. Second, chemical analysis shows that in the dilutions used the calcium concentration of the Ringer is not appreciably altered. Moreover, the parathormone-Ringer solution was so made that on its addition there would be no change in the initial Ca concentration of the perfusing fluid. When this additional care was taken, parathormone still exerted its typical effect.

In conclusion, it may be said that the parathormone effect on the perfused muscle preparation is due to a specific substance intimately related to the principle which raises the blood calcium.[3]

PART III. *The analysis of the parathormone effect.* In the experiments described above, the muscle was invariably stimulated through the nerve, but the same effect is obtained by direct stimulation of the muscle with insertion of electrodes into the muscle tissue. This experiment, however, does not locate the point of action of parathormone.

To investigate this question, three groups of experiments were performed in which the nerve endings were blocked either partially or completely. In the first group curare was used. The muscle was perfused with a Ringer solution containing curare in a dilution of 1:50,000 until a complete curare-effect was obtained. Then a Ringer-curare-parathormone solution was substituted. As the first curve in figure I (plate II) shows, the parathormone effect is absent. The curare was then "washed out" by perfusion with Ringer, and the preparation was tested with parathormone-Ringer

[3] At this point it should be mentioned that experiments undertaken after this paper was written show that parathyroid extract prepared according to the method of Tweedy differs from all the other extracts studied in that it shows no neuromuscular effect while retaining its calcium-raising property. Whether there are two hormones in the ordinary extract, or whether some small chemical change in the molecule impairs its effect on the neuromuscular junction without destroying its calcium-raising power in the intact animal is impossible to state at this time. Further work is being done in order to elucidate these questions.

periodically. After 20 minutes a partial restoration of the parathormone effect was observed (note partial restoration of conductivity at 21 min. point fig. I, plate II); after 30 minutes more of Ringer perfusion, the function of the neuromuscular junction was completely restored and a typical parathormone effect was obtained. This experiment shows that the parathormone effect only occurs when the neuromuscular junction allows the impulses from the nerve to pass to the muscle, and the parathormone effect

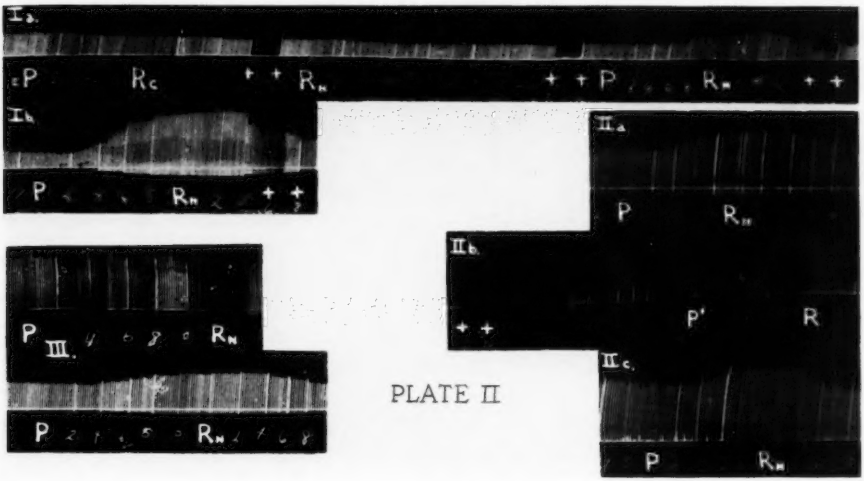


PLATE II

Plate II. Direct stimulation of the muscle. Fig. I. cP = Ringer + curare 1:50,000 + parathormone 1:200. R_C = Ringer + curare 1:50,000. R_N = Ringer. P = parathormone 1:200 in Ringer. At ++ indirect stimulation in order to test the conductivity of the neuromuscular junction.

Fig. IIa. The typical parathormone effect in $CaCl_2$ containing Ringer. IIb. The absence of the parathormone effect in $CaCl_2$-free Ringer. IIc. The repetition of IIa after readministration of $CaCl_2$-Ringer. P = parathormone 1:200 in $CaCl_2$-Ringer (0.075 gm. per liter). P^1 = parathormone 1:200 in $CaCl_2$-free Ringer. R_N = Ringer with 0.075 gram $CaCl_2$ per l. R = Ringer without $CaCl_2$.

Fig. III. Upper curve = denervated muscle. Lower curve = normal muscle. P = parathormone 1:250.

is, other conditions being equal, directly proportional to the conductivity of the neuromuscular junction.

It has been shown recently, by Taubmann and Hikiji (1931), that it is possible to block the neuromuscular junction by lack of calcium in the perfusion fluid. So, in a second group of experiments, absence of calcium was used as a means of blocking the neuromuscular junction. Figure IIa (plate II) shows the typical parathormone effect in Ringer with 0.075 gram

$CaCl_2$ per liter. Then the preparation was perfused with $CaCl_2$-free Ringer; the indirect excitability disappeared completely, but the direct excitability of the muscle was preserved although the height of contraction was diminished. After a uniform height of contraction had been reached the solution was exchanged for one containing parathormone 1:200 in Ca-free Ringer. No influence upon the height of contraction was observed (fig. IIb). By changing back to $CaCl_2$-Ringer the indirect excitability was restored and the preparation reacted to parathormone again in the typical fashion (fig. IIc, plate II). It may be said that in some cases the height of contraction of the muscle on direct stimulation may not be altered at all by lack of calcium. Nevertheless, the reaction to parathormone is absent; but it can be regularly restored by renewed perfusion with $CaCl_2$-containing Ringer.

In a third group of experiments the sciatic nerve was cut on one side and after an elapse of at least 12 days the parathormone effect was studied by direct stimulation of the muscle. As figure III (plate II) shows, the parathormone effect was absent in the legs in which the nerve endings were degenerated (upper curve), whereas in the intact muscle a distinct rise in the height of contraction, due to the administration of parathormone, was observed (lower curve).

These three groups of experiments show conclusively that no matter by what means the neuromuscular junction is blocked, the parathormone effect is absent. If the block is reversible, the effect reappears pari passu with the restoration of the conductivity of the neuromuscular junction, which, therefore, must be considered as the structure upon which the parathormone acts.

A series of systematic studies, in which the $CaCl_2$ concentrations were gradually decreased, showed that the parathormone reaction (by indirect stimulation) became negative at 2 mgm. per cent $CaCl_2$. At 3 mgm. per cent $CaCl_2$ the reaction is extremely small but can be distinctly improved by the addition of $SrCl_2$. Here again a parallelism between neuromuscular conductivity and parathormone reaction is shown, since together with the improvement in the parathormone reaction the height of contraction in indirect stimulation is somewhat increased. These experiments further indicate that $SrCl_2$ may replace $CaCl_2$ to a certain extent in its ability to maintain the conductivity of the neuromuscular junction. Still more striking were experiments in which, in the complete absence of $CaCl_2$, the conductivity of the neuromuscular junction was maintained by $SrCl_2$, although only to a slight extent. Under these conditions the parathormone effect remained present.

Furthermore, it was found that other bivalent cations such as Mg or Ba cannot functionally replace calcium in relation to the neuromuscular junction.

PART IV. *The calcium-parathormone relationship.* Since both calcium and parathormone act on the neuromuscular junction, it is not unreasonable to assume that the efficiency of the calcium may be altered by parathormone. To examine this question numerous experiments were carried out in which the effect produced by a certain amount of $CaCl_2$ in Ringer was compared to that produced by the same amount of $CaCl_2$ in the presence of parathormone in the same nerve-muscle preparation. A

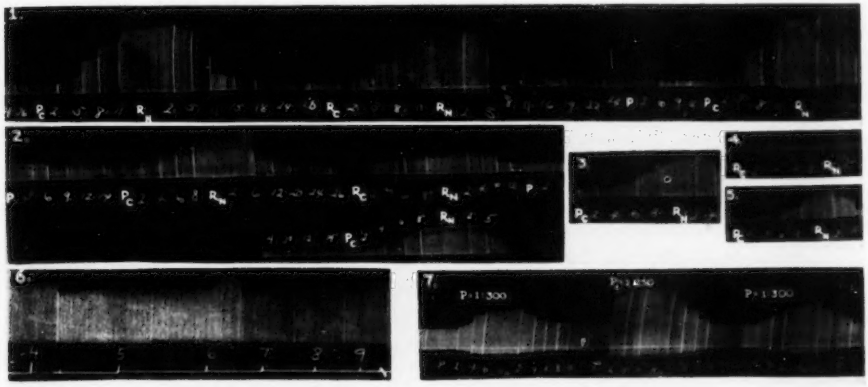


PLATE III

Plate III. Figs. 1-5. The effect of parathormone upon the calcium effect upon the muscle. Figures 1, 2 and 3–5 represent three different experiments. R_N and R_C = Ringer with 0.075 and 0.131 gram $CaCl_2$ per l. respectively. P and P_C = parathormone (1:1000)-Ringer with 0.075 and 0.131 gram $CaCl_2$ per l. respectively. A period of P preceded regularly the period of P_C even where its record had been omitted as in the beginning of figure 1 and in figure 3.

Fig. 6. Straub's heart preparation (R. esculenta). At the mark 0.1 cc. of various dilutions of a $CaCl_2$ 1.2 per cent was added to 2 cc. of Ringer. During the experiments 4, 5 and 6 Ringer containing parathormone 1:1000 was used. The above mentioned $CaCl_2$ solution was diluted as follows: 4 and 7, 1:80; 5 and 8, 1:40; 6 and 9, 1:20.

Fig. 7. A quantitative comparison of the parathormone effect in concentrations of 1:250 and 1:300.

subthreshold concentration of parathormone was chosen (1:1000 is ordinarily about the threshold concentration). Three such experiments are reproduced in plate III. In figure I the first and the third curve show the effect of increased $CaCl_2$ (from 0.075 gm. to 0.131 gm. per liter) in the presence of parathormone (note that parathormone 1:1000 does not alter the height of contraction during 12 minutes), whereas in the middle curve the calcium effect in ordinary Ringer is reproduced. The experiment shows that the calcium effect is increased in the presence of parathormone.

This result is more striking when we take into account the fact that the height of contraction is gradually decreasing throughout the whole experiment. Whereas in figure I the calcium effect in a parathormone-containing solution is identical at the beginning and at the end of the experiment, we find quite often a decline due to the increasing fatigue of the preparation. But under these conditions the striking result that parathormone increases the calcium effect becomes still more impressive (note figs. II–V). In the light of these results it may be said that parathormone sensitizes the neuromuscular junction to calcium and it does so in concentrations in which it does not measurably alter its conductivity.

Since calcium displays numerous other effects than those already described, it seemed of importance to determine whether calcium effects in general can be increased by parathormone or whether this effect is rather specific and perhaps due to the fact that both act upon the same structure. For this purpose the effect of calcium with and without parathormone was studied on the frog's heart by means of the Straub preparation. A typical experiment is reproduced in figure VI of plate III. The amounts of $CaCl_2$ added at 4, 5 and 6 are the same as at 7, 8 and 9 respectively, but in the first group parathormone (1:1000)-Ringer was employed, whereas in the second group ordinary Ringer was used. The effects of calcium on the height of the ventricular contraction are exactly the same regardless of the presence or absence of the hormone. In another group of experiments a much higher concentration of parathormone was used without observing any effect. Thus, in contrast to voluntary striated muscle, heart muscle reacts to $CaCl_2$ in an identical manner regardless of the presence or absence of parathormone.

A final series of experiments was carried out in order to test the effect of calcium on the imbibition of water by striated voluntary muscle, with and without parathormone. The arrangement was similar to that used by Abderhalden and Gellhorn (1922). Fifteen pairs of gastrocnemius and sartorius muscles of R. esculenta were suspended in solution (4°C.) consisting of 5 cc. isotonic NaCl+ 2 cc. isotonic $CaCl_2$ and weighed at various intervals. The experiments were interrupted when the excitability dropped considerably. These results served to indicate the variations of a control series. A similar group of experiments was carried out in which one solution contained parathormone (1:280) and the other none. The pH was 7.14 and 7.08 respectively. The results[4] indicate that the effect of calcium in decreasing the water content of the muscle is not appreciably altered by the presence of parathormone, since the variations occur at random and are of the same order of magnitude as those of the control experiments.

[4] Table omitted to save space.

DISCUSSION. In the first two parts of this paper it has been shown that parathormone regularly exerts a very distinct and reversible effect upon voluntary striated muscle. This effect is proportional to the concentration of the homone and, although the sensitivity of different preparations varies somewhat, the reactivity of the same preparation is rather constant. In view of the fact that previous methods of assay of parathormone are not entirely satisfactory, as Collip and Thomson report, it seems to us that the described muscular reaction may be of considerable value in the assay of parathormone. Figure VII (plate III) illustrates that it is easy to distinguish two extracts, the hormone content of which differs by about 20 per cent, and it seems to be possible to refine the reaction by varying the $CaCl_2$ concentration in the perfusion fluid.

In part III it was shown that parathormone acts upon the neuromuscular junction. It has been stated that prior to the beginning of the experiment the preparation was brought into a state of relative fatigue in which the original height of contraction was greatly reduced upon indirect stimulation. Since we know that the neuromuscular junction is easily fatigued, and since we furthermore worked with solutions of relatively low $CaCl_2$ concentrations, which in the light of Taubmann's and our own observations impair the conductivity of the endplate, we must conclude that only a fraction of the endplates is functioning. Since it has been shown that both $CaCl_2$ and parathormone act on the neuromuscular junction, we must assume that under the influence of parathormone fatigue is decreased because more junctions conduct the impulses arriving from the nerve. Obviously, parathormone increases the excitability of these structures in proportion to its concentration and thereby restores the function of an increasing number of endplates. This hypothesis clarifies the observations on the effect of parathormone on fatigue.

It has been shown that under the influence of parathormone fatigue is greatly delayed. The reason for this is that more endplates withstand fatigue better, just as the endplates of a fresh muscle do in comparison to one which has already been brought into a state of fatigue. Since it has been postulated that calcium and parathormone act upon the neuromuscular junction in a similar manner, it is to be expected that calcium will delay fatigue. This is in fact true as has been shown by Gellhorn (1931–32) under many conditions.

In the light of this interpretation, the increased efficiency of calcium under the influence of parathormone, even if the latter is used in subliminal quantities, is easily understandable as a phenomenon of summation of two stimuli acting upon the same structure, namely, the neuromuscular junction. The observation that such a summation process does not occur in the effect of calcium on the swelling of voluntary striated muscle, does not involve theoretical difficulties, since in this phenomenon the calcium acts

on the colloidal structure of the muscle and not on the neuromuscular junction. Neither is it surprising that the calcium-parathormone summation is not true for the heart, because it is well known that concentrations of curare which paralyse the motor endplates do not affect the heart action and the action of calcium on the heart muscle involves the contractility of the muscle fibers and not a structure similar to the motor endplate.

SUMMARY

In perfusion experiments (frog) it was found that parathormone regularly increases the height of contractions up to as much as 400 per cent, depending on the strength of the concentration used. The effect is reversible. It depends upon the number of "units" contained in the extract. Inactivation of parathormone by boiling with 10 per cent HCl or ethyl alcohol destroys both the blood calcium-raising principle and the described muscular reaction.

Parathormone is shown to act on the neuromuscular junction, since its typical reaction upon the muscle is abolished by curare, lack of calcium, and degeneration of the nerve endings. Calcium can be replaced partially by strontium.

The effect of calcium on the muscle is increased by subliminal concentrations of parathormone, but such a synergism is absent in the heart. The decrease in imbibition caused by $CaCl_2$ is not enhanced by parathormone.

The mechanism of these findings is discussed. The action of parathormone upon the skeletal neuromuscular junction is recommended as a criterion for the assay of parathormone.

The author wishes to acknowledge his indebtedness to Miss Joan Osten for her valuable technical help.

REFERENCES

ABDERHALDEN, E. AND E. GELLHORN. Pflüger's Arch. **196:** 584, 1922.

GELLHORN, E. Biol. Bull. **60:** 383, 1931.
This Journal **100:** 447, 452, 1932.
This Journal **105:** 353, 1933.

SCHMITT, O. H. AND F. O. SCHMITT. Science **76:** 328, 1932.

TAUBMANN, G. AND K. HIKIJI. Arch. f. Exp. Path. u. Pharmakol. **161:** 621, 1931.

THOMSON, D. L. AND J. B. COLLIP. Physiol. Reviews **12:** 309, 1932.

TRENDELENBURG, P. Die Hormone, vol. II. Berlin, J. Springer, 1934.

TWEEDY, W. R. J. Biol. Chem. **88:** 649, 1930.

THE METABOLISM OF SINGLE NORMAL MOUSE LYMPH NODES

JOSEPH VICTOR

From the Department of Pathology, College of Physicians and Surgeons, Columbia University, New York, and the Department of Genetics, Carnegie Institution of Washington, Cold Spring Harbor, Long Island

Received for publication December 7, 1934

During the course of investigations on experimental leukemia in mice, it became important to study the metabolism of single lymph nodes removed from the same mouse at different times. A single normal lymph node in the mouse weighs from 2 to 5 mgm. (moist weight). Usual respirometers for study of oxygen consumption and aerobic and anaerobic glycolysis are not suitable for such small quantities of tissue because of the relatively large effects of temperature variations. Therefore a new respirometer has been developed and its operation checked by comparisons with larger amounts of similar material in the apparatus previously used.

APPARATUS. The apparatus is a modification of the constant pressure differential volumeter as used by Fenn (2). It consists of two chambers separated by a capillary in which a drop of kerosene records the volume changes. If the volumes of the two vessels are equal, the movement of the drop is one-half of the actual volume change. But by greatly reducing the volume of the experimental bottle in comparison with the control bottle, the movement of the drop can be made to approach the total volume change. For example, with a difference of 300 times in bottle volumes, the volume change in the capillary can be measured practically directly, the correction factor being 1/301 of the total volume changes. The sensitivity of the apparatus then depends upon the capillary bore, namely, the distance a drop in the capillary must travel to take up a given volume change. The sensitivity can be increased by optical magnification of the drop (5). Gerard (3) has used the above principle in measuring the respiration of nerve due to natural impulses.

The stability of the apparatus is increased for several reasons. A small volume minimizes thermal irregularities. Variation in tensions at the meniscus drop and at the fluid glass interface are decreased as the chamber volume is decreased. The whole system is a differential one.

More specifically, the experimental vessel consisted of a hollow chamber, *C*, within a stopcock (fig. 1). The chamber was a small bubble with

a flat bottom and a gas space of about 0.1 cc. It had two openings near the top at an angle of 90° to each other. One opening, *O*, was 2 mm. in diameter, the other, *P*, 1 mm. The openings communicated with a capillary, *K*, about 0.5 mm. in diameter, and a tube at a right angle to the capillary. In the other part of the stopcock, where it joined the capillary, was a small dilatation in the capillary, *D*, about 1.5 mm. in diameter.

The capillary was 10 cm. long. At one end was a ground joint for joining the capillary with the large control flask. The latter had a volume of about 30 cc. At its further end was a two-way stopcock, *L*. The

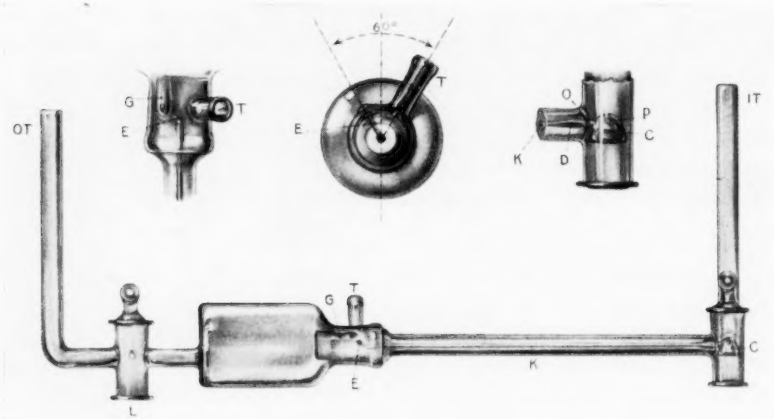


Fig. 1. *C* = chamber in stopcock for tissue and solution. *D* = dilatation in capillary of outer portion of stopcock for alkali. *P* = 1 mm. opening in chamber of stopcock. *O* = 2 mm. opening in chamber of stopcock for insertion of tissue and solutions. *K* = capillary. *E* = capillary connection in ground joint. *G* = groove in outer part of ground joint attached to large flask. *T* = tube in outer part of ground joint attached to large flask. *L* = two-way stopcock. *IT* = tube for introduction of gas. *OT* = outlet tube.

ground joint had a longitudinal groove, *G*, for connecting the capillary to the flask, and a side tube, *T*, communicating with the outside. The capillary could join the side tube by rotating it through an angle of 60° from the groove, *G*. The side tube had an inside diameter of about 2 mm. and was about 1.5 cm. long. By means of this ground joint with its groove and side tube, any gas could be introduced into the experimental chamber and capillary. After introducing the special gas, the indicator, a drop of kerosene, could be placed in the capillary without contaminating the gas with air.

Procedure. About 0.05 cc. of Ringer's phosphate solution (for O_2

consumption measurements) pH 7.44 (38°) containing 10 mgm. P per 100 cc. and 0.2 per cent glucose were placed in the stopcock (*S*) by means of a capillary pipette through the 2 mm. opening. Special care was taken to avoid wetting the opening. Pipettes with fine curved tips were convenient for this. Then, one or two slices of the lymph node, about 1 mgm. moist weight, were placed in the fluid. The tissue was weighed to 0.02 mgm. on a Roller-Smith torsion balance. For absorbing CO_2, a narrow strip of filter paper was placed in the dilated portion of the capillary, *D*, and moistened with 0.2 N NaOH. The alkali was close enough to the tissue to allow rapid absorption of the CO_2 produced. A drop or two of Ringer's solution was placed in the control bottle, enough to exert a definite vapor pressure. Then oxygen, in the experiments for oxygen consumption measurements, or 95 per cent O_2 — 5 per cent CO_2 or 95 per cent N_2 — 5 per cent CO_2, in the case of aerobic or anaerobic glycolysis determinations, was flushed through the chamber and capillary by running it into the inlet tube, *I. T.* In the glycolysis measurements the procedure was similar to Warburg's (9). The Ringer's solution contained 2.5×10^{-2} molar $NaHCO_3$ in the control respirometer and in the respirometers used for glycolysis measurements, 0.2 per cent glucose in addition. The capillary was connected to the control bottle while the gas was flushed through the experimental chamber. The optimum rate was about 10 cc. per minute. At this rate the gas pressure did not drive the solution out of the chamber. Evaporation was avoided by bubbling the gas through the solutions used before flushing the experimental chamber. This was done through a wash bottle connected in series with the respirometer. After ten minutes, the gas pressure was shut off. The control bottle was rotated so that a drop of kerosene could be placed in the side tube, *T*. Care was taken to introduce the kerosene so that no air was present beneath it. With further rotation of the capillary, the kerosene ran into the capillary. By proper adjustment of the capillary beneath the side tube, the kerosene could be introduced without any air. The capillary was again connected with the control bottle and by pressure on the outlet tube, *O. T.*, the drop could be placed into any position in the capillary.

Then the stopcock, *S*, was turned so that only one opening, preferably the 2 mm. one, communicated with the capillary, the inlet tube, *I. T.*, being shut off from the chamber. The apparatus was placed in a water bath and allowed to come to equilibrium. About 10 or 15 minutes were necessary to bring this about when the temperature change was that of the room (about 22°) to a water bath with a temperature of 37.5°. The stopcock, *L*, attached to the control chamber was shut off and readings begun.

This arrangement with all parts of the apparatus exposed to the water bath permits rapid temperature equilibrium. Using mammalian tissues

at 37.5°C., it is unnecessary to magnify the movements of the drop, since the rate of oxygen consumption is high enough in the case of most tissues to produce sufficient deflection. A deflection of about 7 mm. per mgm. (moist weight) per hour is observed when the capillary diameter is 0.5 mm. and the Q_{O_2} is 6.0.

The respirometers are shaken 120 to 140 times a minute. Optimum results are obtained at this rate. The temperature was 37.5°C. ±0.005°.

CALCULATIONS. The vessel constant, K.

$$K = b \times c \times \frac{BP - VP}{760} \times \frac{273}{t + 273}$$

Where $b = \frac{a + 1}{a}$ and $a = \frac{\text{Gas volume of control bottle}}{\text{Gas volume of experimental bottle}}$

c = capillary volume per cm.

BP = barometric pressure in mm. Hg.

VP = vapor pressure in mm. Hg at t degrees centigrade.

t = degrees centigrade.

Then, the $Q_{O_2} = \frac{K \times \text{deflection} \times c}{\text{Mgm. (dry weight) per hour}}$

The same constants are used for obtaining the $Q^{O_2}_{CO_2}$, cmm. CO_2 equivalent to acid produced aerobically per mgm. per hour and the $Q^{N_2}_{CO_2}$, cmm. CO_2 equivalent to acid produced anaerobically per mgm. per hour.

RESULTS. Series 1 of table 1 shows the mean metabolic rates of 24 single axillary lymph nodes of mice, biopsied under ether anesthesia. The mice were of strain C58, between the ages of 6 to 8 weeks.

Table 1 is a summary of the mean Q_{O_2}, $Q^{O_2}_{CO_2}$, and $Q^{N_2}_{CO_2}$ of single lymph nodes compared with the metabolic rates for mice of the same description previously observed (7, 8) in apparatus requiring the combined lymph nodes of four mice for each observation. The $Q^{O_2}_{CO_2}$ and $Q^{N_2}_{CO_2}$ for the two series are not significantly different, being only 1.5 × their probable errors, but the rates for oxygen consumption differ. The difference of Q_{O_2} is 6.5 times its probable error and thus statistically significant. This does not necessarily mean that the difference in apparatus was responsible, for one essential difference existed in the experimental procedure of these two series, namely, ether anesthesia was used during the removal of the biopsied lymph nodes, while in the other series the mice were killed by pressure on the cervical vertebrae.

That the difference in Q_{O_2} was in fact due to the ether and not to the new technique has been shown by using ether with the previous technique. Eleven mice of the same strain and ages as before were killed by ether inhalation. The combined lymph nodes of each mouse were sectioned and placed in Ringer's solution in Fenn respirometers as employed in the previous experiments. The rates of oxygen consumption of these lymph nodes (series 3, table 1) do not differ significantly from the rates for single

lymph nodes, but they are much less than those observed for lymph nodes removed from mice killed by pressure on the cervical vertebrae (series 2, table 1). Thus ether anesthesia in mice does decrease the rate of oxygen consumption of lymph nodes removed from them. This is in accord with the findings that ether decreases the CO_2 production of animals (4), brain (1), and isolated nerves (6). On the other hand, the biopsy experiments (table 1, series 1) show that although the respiratory rate of normal lymph nodes is decreased by ether anesthesia, the aerobic and anaerobic glycolysis are unaffected.

TABLE 1

Differences in metabolism of lymph nodes due to different methods

The only difference observed was due to ether anesthesia

	Q_{O_2}			$Q_{CO_2}^{O_2}$			$Q_{CO_2}^{N_2}$		
	Mean	S.D.	C.V.	Mean	S.D.	C.V.	Mean	S.D.	C.V.
(1) Present method.....	4.6 ±0.08	0.52	11.3	1.92±0.09	0.69	36.0	6.28±0.17	1.13	18.0
(2) Larger sample method..	5.45±0.11	0.57	10.5	2.13±0.11	0.55	25.8	5.78±0.3	1.54	26.6
(3) Larger sample method with ether anesthesia.....	4.29±0.18	0.91	21.1						
Difference of means		Diff. / P.E. Diff.			Diff. / P.E. Diff.			Diff. / P.E. Diff.	
(1)-(2)	*−0.85*±0.13	*6.5*		−0.21±0.14	1.5		0.50±0.34	1.5	
(1)-(3)	0.31±0.20	1.5							
(2)-(3)	*1.16*±0.21	*5.5*							

SUMMARY AND CONCLUSIONS

1. A new constant pressure differential volumeter is described for measuring the respiration of less than 1 mgm. (moist weight) of tissue. The results have been checked with those obtained with other apparatus in which larger quantities of tissue have been studied.

2. Ether anesthesia decreases the respiratory rate, but not the aerobic or anaerobic glycolysis of normal lymph nodes of mice.

I am indebted to Miss Margaret R. Prest for her assistance in these experiments.

REFERENCES

(1) ALEXANDER, F. G. AND S. CSERNA. Biochem. Ztschr. **53**: 100, 1913.
(2) FENN, W. O. This Journal **80**: 327, 1927.

(3) GERARD, R. W. AND H. K. HARTLINE. J. Cell. and Comp. Physiol. **4**: 141, 1934.
(4) IRWIN, M. J. Gen. Physiol. **1**: 209, 1918.
(5) SCHMITT, F. O. This Journal **104**: 303, 1933.
(6) TASHIRO, S. AND H. S. ADAMS. This Journal **33**: Proc. xxxviii, 1914.
(7) VICTOR, J. AND M. R. WINTERSTEINER. Am. J. Cancer **22**: 561, 1934.
(8) VICTOR, J. AND J. S. POTTER. J. Exp. Med. **60**: 547, 1934.
(9) WARBURG, O. Metabolism of tumours. Constable and Co., Ltd., London, 1930.